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Clinical Interpretation of Laboratory Tests

CLINICAL INTERPRETATION
of
LABORATORY TESTS

by

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Dedicated
to
my children

THELMA GOODALE
and
RAYMOND B. GOODALE

Preface to Second Edition

In publishing a medical book, the author and publisher assume the responsibility of keeping the material up-to-date. In this case, the responsibility has been emphasized by the gratifying reception of the first edition.

Advances in the various fields of medicine since the publication of the first edition have required a thorough study of the text and pertinent literature. The revision has involved restatement, alteration, and expansion in order to embrace these advances. Some completely new material has been added—the subject of potassium metabolism, for example. In connection with this, the conditions that cause decreased and increased serum potassium levels have been discussed.

Where possible, the author has included changes suggested by friends and by reviewers. It is by such helpful suggestions that a book is improved with each edition. In reflecting on the sales reports, it appears that the book has been widely useful, particularly to the general practitioner, the internist, and the surgeon.

The general format has been maintained. Part 1 deals with the normal values and significance of abnormal values of the various body fluids and excreta. Part 2 takes up diseases of various organs by systems with the pertinent laboratory findings. The author has adhered to the brevity and simplicity that characterized the first edition, since these features have drawn favorable comment and have contributed much to the practical value of the book in the hands of its readers.

Again I wish to thank Miss Agnes O'Hare, who prepared the manuscript for this as well as for the first edition.

I am also deeply indebted to the publishers for suggestions and advice given at various times in the preparation of this revision.

Raymond H. Goodale, M.D.

Preface to First Edition

The numerous advances in all branches of clinical pathology have created a demand for ready information on interpretation of laboratory tests and on the most helpful laboratory procedures applicable to the diagnosis of a given disease. It is hoped that this book will fill such a need by bringing together the laboratory and the clinic. Medical students, interns, and medical technologists, as well as clinicians, should find it a valuable reference-work for their particular problems in this field.

Part 1 deals with the physiology, normal values, and the significance of abnormal values of the various body fluids and excreta. This section also includes chapters on basal metabolic rates, liver-function tests, bacteriologic, virus, and mycotic examinations, skin tests, and poisons. Chemotherapy and antibiotic therapy in relation to bacteriology are discussed in Chapter 13, Bacteriologic Examinations.

In Part 2, diseases with associated laboratory findings are discussed according to the various systems, e.g., the Blood and Bone Marrow. Each disease which lends itself to laboratory diagnosis is briefly outlined. This is followed by a list of changes to be expected in the pertinent laboratory examinations. These examinations are given in the order of importance, with the most important first.

Although it is hoped that other atomic bombs will not be used, the blood changes resulting from those dropped on Japan are listed.

These sequelae are similar to those which have been seen in radiation sickness following overexposure to x-rays.

Part 2 ends with methods of preparing body fluids, excreta, and tissues for the laboratory. The proper methods of preparing specimens is important in order that accurate results may be obtained. For example, the blood glucose level drops when it is kept at room temperature unless a preservative is added.

Although certain tests are pathognomonic, clinicians should not rely entirely upon the laboratory for a diagnosis. The laboratory and x-ray department are helpful adjuncts in the modern doctor's diagnostic armamentarium, but they should not replace a careful history and physical examination. The history and physical examination are also important so that the laboratory tests requested will be pertinent to the diagnosis. Certain routine tests are desirable for the protection of the patient because certain diseases may be symptomless at the time. Thus, most hospitals perform routine urinalyses, blood-count estimations, and serological tests for syphilis. However, it should be remembered that frequent venipunctures may work a hardship on the patient.

Dr. Alexander S. Wiener was kind enough to help me in organizing the material on blood groups and the Rh factor. For this, I am most appreciative. Dr. William T. Carleton reviewed Chapter 12, Liver-Function Tests, and made several valuable suggestions.

I wish to thank Mrs. Isabel Baker Carleton for her painstaking work in preparing certain illustrations. The original photographs were taken by Mr. Matthew D. Carrigan, who has always been most cooperative.

Here I wish to express my gratitude to my secretary, Miss Agnes M. O'Hare, who spent many hours in preparing the manuscript and in checking references.

Finally, I wish to express my appreciation to the publishers for their help in the many details about which I have asked their advice.

Raymond H. Goodale, M.D.

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PART 1

Body Fluids, Excreta, and Functional Tests

CHAPTER 1

Hematology

BONE MARROW

Introduction:

The functioning bone marrow is made up of young and adult blood cells and their precursors, blood vessels, fat cells, and reticulum. Almost all of the bone marrow is red, and is functioning during the first few years of life. From five years of age, fat cells appear in the marrow of the long bones so that ultimately hematopoietic marrow is found only in the proximal ends of the femur and humerus. The red marrow in adult life is limited mostly to the bones of the trunk and head; namely, the skull, vertebrae, ribs, sternum, and innominate bone. In adults, the total bone marrow has been estimated to weigh from 1600 to 3700 gm. (3½ to 8 pounds). However, only about half of it is normally in an active state.

The nonfunctioning marrow of the long bones retains the essential hematopoietic structures; and with proper stimulation, it will start to function. This is seen in pernicious anemia, myelogenous leukemia, and to a certain extent during residence at high altitudes. As a result, the fat is gradually replaced by red marrow.

Extramedullary hematopoiesis may on occasion supplement the formation of cells by the bone marrow. Foci of hematopoiesis have been seen in the spleen, lymph nodes, adrenals, cartilage, broad ligament, organizing thrombi, and fat tissue. These foci occur particularly in anemia of infants and young children,[1] pernicious anemia,[2,3] macrocytic anemia of liver disease,[4] metastatic carcinoma,[1,5] malignant granuloma (Hodgkin's disease) of the bone marrow,[4] bone-marrow injury by a toxin,[4] anemia resulting from severe sepsis,[1,6] and leukemia.[5,7] These blood-forming foci may be made up of erythroblasts or their precursors, myelocytes, megakaryocytes, or all three types of cells.

Function of Bone Marrow:

The bone marrow forms erythrocytes, granulocytes, and platelets. These cells are formed from special precursor cells, and normally enter the blood stream only after they have matured. The method whereby the cells enter the circulation is still debated. However, in a normal person the bone marrow supplies sufficient numbers of blood cells to keep pace with normal destruction. Sabin[8] found that granulocytes enter the blood stream at regular intervals with small hourly increases and larger daily increases in the afternoon and at midnight. Larger numbers of granulocytes enter the blood stream when the chemotactic influence of an infectious process is present. It is thought that anoxemia is the basic stimulus which calls forth greater numbers of erythrocytes. Anoxemia is caused by anemia, inadequate oxygenation of erythrocytes, fixation of hemoglobin as in carbon monoxide poisoning, and living in high altitudes where oxygen tension is lower.

Bone-Marrow Biopsy:

Bone-marrow biopsies are usually secured from the sternum because of its accessibility and because active bone marrow may be obtained at all ages. The upper part of the body of the sternum is usually chosen because it does not give as readily when pressure is applied. Biopsies may be taken by trephining or by sternal puncture. The latter procedure is simpler and not so much of an ordeal for the patient.

The technic of sternal puncture was introduced by Arinkin[9] in 1927. The skin is carefully sterilized, and aseptic technic is used throughout. The skin, subcutaneous tissue, and periosteum are anesthetized with 1 per cent solution of procaine. A short, beveled, 18-gauge needle with a lumen of from 1 to 2 mm. is employed. The needle should be equipped with an adjustable guard. In penetrating the body of the sternum, the guard should be set at 1 cm. for an adult and from 0.6 to 0.2 cm. for a child.

TABLE 1: PERCENTAGES OF CELLS IN BONE MARROW OF NORMAL ADULTS*

	Range	*Average*
Myeloblasts	00.3 to 05.0	02.0
Promyelocytes	01.0 to 08.0	05.0
Myelocytes:		
Neutrophilic	05.0 to 19.0	12.0
Eosinophilic	00.5 to 03.0	01.5
Basophilic	00.0 to 05.0	00.3
Metamyelocytes ("juvenile" forms)	13.0 to 32.0	22.0
Polymorphonuclear neutrophils	07.0 to 30.0	20.0
Polymorphonuclear eosinophils	00.5 to 04.0	02.0
Polymorphonuclear basophils	00.0 to 00.7	00.2
Lymphocytes	03.0 to 17.0	10.0
Plasma cells	00.0 to 2.0	00.4
Monocytes	00.5 to 5.0	02.0
Reticulum cells	00.1 to 2.0	00.2
Megakaryocytes	00.03 to 03.0	00.4
Pronormoblasts (macroblasts)	01.0 to 08.0	04.0
Normoblasts (basophilic, polychromatophilic, and acidophilic)	07.0 to 32.0	18.0

* Wintrobe, M. M.: Clinical Hematology. Lea & Febiger, Philadelphia, 1946, p. 48.

When anesthesia is effective, insert the needle into the center of the bone with a boring motion. When the cortex is pierced, a give is felt as the point encounters the soft marrow. The stylet is removed, and a 2 or 5 cc. syringe is attached. Suction is then applied, and marrow pulp is pulled up into the syringe. If no specimen is visible in the syringe, the stylet is replaced, and the needle is forced deeper into the marrow. Failing in this procedure, 0.5 cc. of sterile isotonic saline solution is injected into the marrow to loosen the tissue. Suction is again applied with the syringe. After removal of the needle, a sterile bandage is placed over the puncture wound.

Smears, contact smears, and supravital preparations may be made from the material. Satisfactory tissue for sectioning is not usually obtained by this method, but small bits of tissue may be fixed and blocked together. The smears may be stained with Wright or Giesma stains.

Sternal puncture is contraindicated in serious hemorrhagic conditions, including hemophilia.

Normal Bone Marrow (Plate I):

Erythrocyte counts and leukocyte counts may be made on marrow material, but there are marked fluctuations. The differential count yields the most information. For a satisfactory differential count, at least from 300 to 500 cells must be counted.

Bone-Marrow Changes in Disease:

Careful microscopic examination and intelligent interpretation of bone-marrow smears yield information in certain blood disorders in which the peripheral blood shows little or no change. Aleukemic leukemia may be proved by the presence of increased numbers of myeloblasts and more immature forms. They are helpful in differentiating pseudoleukemia from true leukemia, pseudopernicious anemia from true pernicious anemia, and granulocytopenia due to marrow aplasia from granulocytopenia associated with drug sensitivity. They are also helpful in diagnosing aplastic anemia and occasionally may show cells of primary or secondary bone tumors.[10,11]

Kolmer[12] has summarized the important changes in many of these diseases as follows:

Disease	*Important Changes*
Pernicious and related anemias (see p. 345)	Untreated or during relapse: (1) increase of nucleated erythrocytes; (2) preponderance of megaloblasts; (3) increase of reticulum (Ferrata) cells; (4) abnormal leukopoiesis, especially lymphocytes; (5) reduction in megakaryocytes

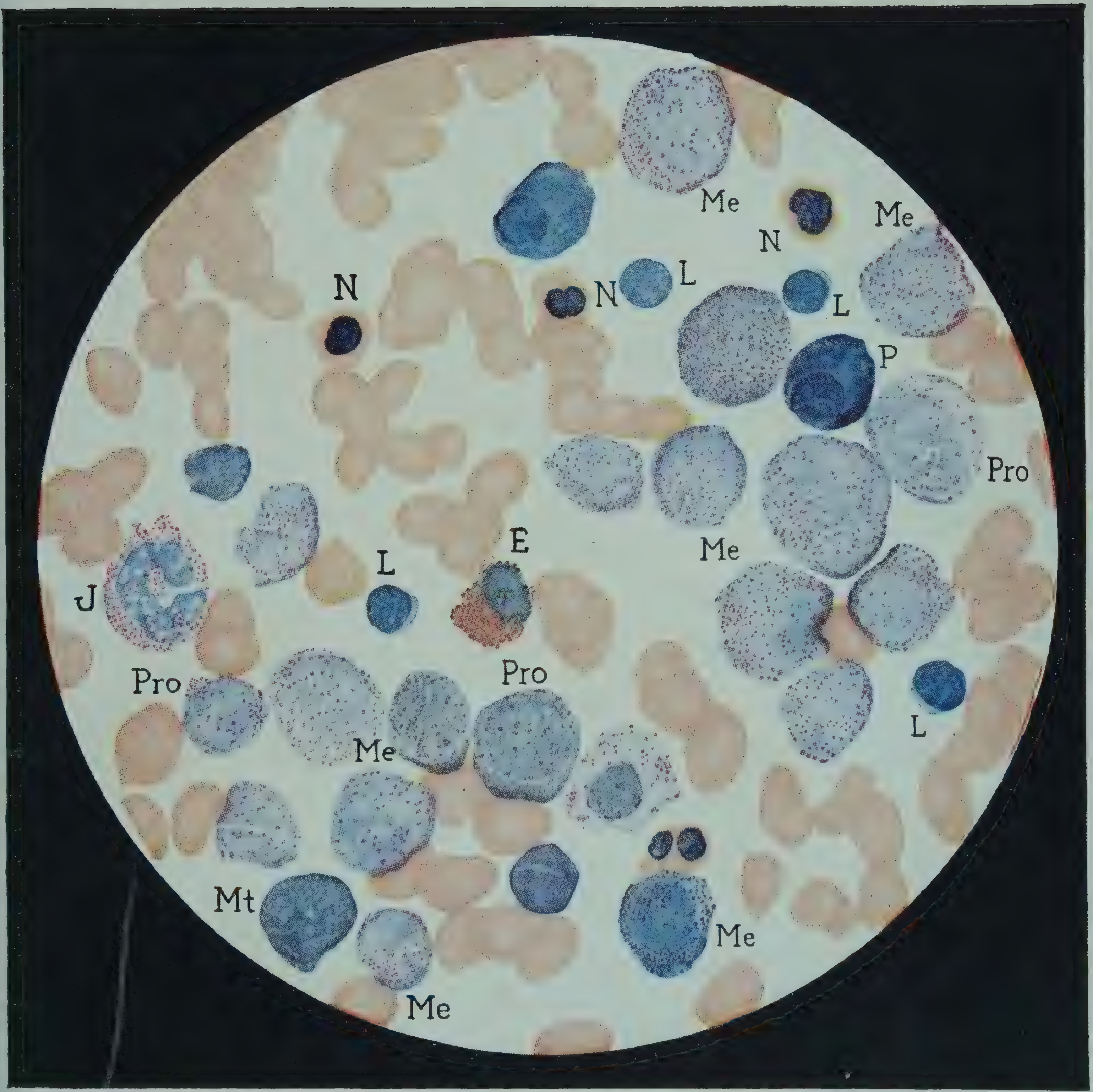
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Plate I: Smear of bone marrow as obtained by sternal puncture. (From Tice: Practice of Medicine. W. F. Prior Co., Hagerstown, Md.)

Disease	*Important Changes*
Aplastic anemia (see p. 353)	Chiefly red blood corpuscles: relative lymphocytosis constituting from 60 to 100 per cent of the nucleated cells; striking immaturity of the red and white corpuscles; they may be normally cellular or hyperplastic
Acute hemolytic anemias (see pp. 330–332)	Markedly hyperplastic: 60 per cent or more of the nucleated cells belong to the erythrocytic series; leukocytes relatively reduced
Chronic hemolytic anemias (see pp. 332–334)	Normoblastic hyperplasia characteristic (normoblasts or macroblasts): no megaloblasts
Sickle-cell anemia (see p. 338)	Largely nucleated red cells (chiefly normoblasts): there may be moderate shift to the left of the myeloid leukocytes; eosinophils relatively increased; megakaryocytes may be increased
Hypochromic microcytic anemia (see pp. 342–345)	Hyperplastic: increase of normoblasts; no megaloblasts; granulopoiesis usually normal
Congenital hemolytic jaundice (see pp. 332-333)	Erythropoietic hyperplasia of the normoblastic type: no megaloblasts or giant, abnormal leukocytes
Thrombopenic purpura (see p. 361)	Many megakaryocytes: usually an increase of erythroid elements due to severe hemorrhage and anemia
Erythremia (see p. 365)	Dark red and very cellular: hyperplasia of all elements, moderate increase of nucleated erythrocytes; sometimes an increase of megakaryocytes, myelocytes, and myeloblasts
Myeloblastic and myelocytic leukemia (see pp. 368–371)	Marked hyperplasia: crowded with myeloblasts and more primitive cells; in eosinophilic leukemia, preponderance of eosinophils; in monocytic leukemia, myelocytes and myeloblasts; also "monoblasts" and "monocytes" in some cases; in chronic myelocytic leukemia, the differential count is similar to that of the blood
Lymphocytic leukemia (see pp. 371–373)	May be only slightly changed: usually, however, a well-marked lymphocytosis (30 to 90 per cent of the cells)

Disease	*Important Changes*
Aleukemic leukemia (see p. 374)	Of great value in diagnosis: frequently the changes are identical with those of typical blood findings; sometimes misleading when only a few cells are obtained, but pronounced immaturity of the leukocytes may be observed when cellularity is reduced; in leukopenic cases of lymphocytic leukemia, marrow lymphocytosis may be slight or absent
Infectious mononucleosis (see p. 379)	Increase of lymphocytes or moderate shift to left of myeloid leukocytes: chiefly of value from a negative standpoint in the sense that findings characteristic of leukemia are absent
Agranulocytosis (see p. 375)	Normal erythropoietic tissue and normal numbers of megakaryocytes: striking lack of granulocytes; plasma cells, lymphocytes, and reticulum cells may be increased
Malignant granuloma (Hodgkin's disease) (see p. 380)	Findings variable and nonspecific: there may be slight shift to left in the myeloid cells; also slight monocytosis or moderate eosinophilia; no lymphocytosis; relative reduction in nucleated red cells
Multiple myeloma (see p. 381)	Various types described such as myeloblastic, lymphoblastic, and erythroblastic, with "plasma cells" the usual designation: myeloma cells most characteristic, constituting from 5 to 65 per cent of all cells present

BLOOD

Composition of Blood:

The blood is a fluid in which cells are free and suspended. The fluid part is called "plasma" before clotting occurs and "serum" after clotting occurs. Plasma is made up of water, from 91 to 92 per cent, and solids from 8 to 9 per cent. The solids consist of serum albumin, serum globulin, fibrinogen, sodium, calcium, potassium, magnesium, phosphorus, the nonprotein nitrogen group, neutral fats, phospholipids, glucose, cholesterol, oxygen, and carbon dioxide. The plasma also contains such substances as antibodies, complement, hormones, and enzymes. In adults, the plasma constitutes from 53 to 58 per cent of the blood volume.

The cellular part of the blood consists of leukocytes, erythrocytes, and platelets. As determined by centrifugation, these cells constitute 47 per cent of the total blood volume in males and 42 per cent in females.

Blood Volume:

In normal human adults, the total blood volume ranges from 60 to 88 cc. per kilogram of body weight, depending on the method used for its determination. It represents from 6 to 8 per cent of the total body weight. The blood volume remains relatively constant in spite of fluids taken by mouth or intravenously. Following transfusions, the volume is increased only temporarily because the excess fluid part of the blood is removed by the kidneys and taken up by the tissues.

There is an increase in blood and plasma volume in pregnancy, which, according to Dieckmann and Wegner,[13] is 23 per cent increased blood volume and 25 per cent increased plasma volume at term.

Immediately following hemorrhage, there is a reduction in total blood volume, but the plasma is quickly restored by passage of fluid from the tissues to the blood stream. The cells are replaced in part by the release of blood cells from the storage depots. In excessive hemorrhage, these mechanisms do not suffice, and it is necessary to give one or more transfusions to replace the blood.

Origin of Blood Cells:

It is generally conceded that the bone marrow is normally the site of origin of erythrocytes, granulocytes, platelets, and on occasion lymphocytes. Lymphocytes usually originate from the lymphoblast, which is found in lymph glands, spleen, thymus gland, Peyer's patches, and tonsils. Monocytes, according to the polyphyletic school, originate from a multipotential cell in the bone marrow.

As discussed under Bone Marrow (p. 4), extramedullary hematopoietic sites may develop in certain diseases. These sites may contain erythroblasts or their precursors, myelocytes, megakaryocytes, or all three types of cells.

Functions of Blood:

The blood has numerous functions, which under normal conditions maintain proper body metabolism. These functions are listed by Best and Taylor[14] as follows:

Respiratory: The transportation of oxygen from the air in the lungs to the tissues, and of carbon dioxide from the tissues to the lungs.

Nutritive: The conveyance of food materials, glucose, amino acids, and fats from the alimentary canal to the tissues.

Excretory: The removal of waste products of metabolism, for example, urea, uric acid, creatinine, and so on.

Maintenance of Water Content of Tissues: Though the blood itself is contained within vascular channels, a constant interchange of fluid through the vessel walls takes place. This fluid, which has left the blood vessels and has come into direct contact with the tissue cells, is known as the "lymph," or tissue juice. It closely resembles the blood fluid in chemical composition. The final stage in the transportation of oxygen and food materials to the tissues, and the first stage in the journey of carbon dioxide and waste products from the tissues, are made through the medium of the transuded fluid. It is the very high solvent and ionizing powers of water, of which the blood fluid and lymph are chiefly constituted, which make these such admirable media for the carrying out of the complex chemical processes of the body.

Body-Temperature Regulation: The body owes its ability to regulate its temperature largely to the water of the blood and the tissue fluids. Water possesses three qualities that fit it preeminently to fulfill this purpose:

HIGH SPECIFIC HEAT: The specific heat of water is considerably higher than that of any other liquid or solid. Because of this great heat-storage power of water, sudden changes of body temperature are avoided. Even a cold-blooded animal, such as the frog, has—because of this purely physical quality—some ability to maintain a relatively constant body temperature against transient fluctuations in environmental temperature. A man of average weight develops 3000 Calories in twenty-four hours. This amount of heat is capable of raising the temperature of his tissues (which are mostly water) only about 89.60° F. (32° C.). Heat elimination (radiation and so on) is able to keep pace with heat production, and the body temperature varies but slightly within normal limits. It has been pointed out by L. J. Henderson, however, that if the tissues had the low heat-storage capacity (specific heat) of most substances, an amount of heat equal to 3000 Calories would raise the temperature of the tissues and fluids of the body by from 212° to 302° F. (100° to 150° C.).

HIGH CONDUCTIVITY: The thermal conductivity of water is greater than that of any other ordinary liquid. The advantage of this in the dissipation of heat from deeply situated regions of the body is obvious.

HIGH LATENT HEAT OF EVAPORATION: More heat is required for the vaporization of water than for that of an equivalent amount of any other liquid. About 0.6 Calorie (large) is required for the vaporization of 1 cc. of water. This figure is 50 per cent higher than that of water's closest competitor. Fluid is constantly lost from the body through evaporation from the lungs and skin. A large amount of heat is lost in the process.

These physical properties of water, which make it ideal as a heat-regulating medium, are enhanced by other purely physiological factors. The mobility of the blood and the readiness with which it may be quickly redistributed in the body, combined with the unique physical properties of the fluid itself, make it highly efficient as a regulator of body temperature. The blood may, in a moment, be brought from deeper to superficial regions and spread out in fine vessels over a broad area just beneath the skin. In this way, it greatly increases the radiation of heat. At another instant, in order that heat may be conserved, the fluid is drained from the surface areas and collected in the deeper parts of the body, in the internal organs, muscles, and so on.

Protective and Regulatory: The blood and lymph contain certain chemical substances of a complex nature, antitoxins, lysins, and other antibodies, which are the basis of the body's defense against injurious agents of various kinds. The circulating fluids also bring the endocrine principles from the ductless glands into direct contact with the cells of the tissues.

TABLE 2: SUMMARY OF NORMAL BLOOD VALUES

Erythrocytes (per cu. mm.)	Under two weeks of age	5,100,000 to 5,660,000	
	One to 15 years	4,600,000 to 4,700,000	
	Adult females	4,600,000 to 4,800,000	
	Adult males	5,400,000 to 5,800,000	
Reticulocytes	0.5 to 1.5 per cent of erythrocytes		
Volume of packed erythrocytes	Infants: 49 to 54 (±10) cc. per 100 cc. of blood		
	Children: 35 to 39 cc. per 100 cc. of blood		
	Adult males: 47 (±7) cc. per 100 cc. of blood		
	Adult females: 42 (±5) cc. per 100 cc. of blood		
Mean corpuscular volume	Infants: 84 cu. microns		
	Children: 72 cu. microns		
	Adult males: 82 cu. microns		
	Adult females: 86 cu. microns		
Hemoglobin	Infants: 12 to 14 gm. per 100 cc. of blood		
	Children: 12.5 to 13.5 gm. per 100 cc. of blood		
	Adult males: 16 (±2) gm. per 100 cc. of blood		
	Adult females: 14 (±2) gm. per 100 cc. of blood		
Color index	1 (in absolute terms)		
Mean corpuscular hemoglobin	Children: 26 to 28 micromicrograms		
	Adults: 29 (±2) micromicrograms		
Mean corpuscular hemoglobin concentration	Children: 32 to 34 gm. per 100 cc.		
	Adults: 34 (±2) gm. per 100 cc.		
Saturation index	0.9 to 1.2 (in absolute terms)		
Sedimentation rate	*Adult Males*	*Adult Females*	
(mm. fall in one hour)	0 to 8 mm.	0 to 10 mm. (Cutler)	
	0 to 15 mm.	0 to 20 mm. (Westergren	
	0 to 6.5 mm.	0 to 15 mm. (Wintrobe)	
Fragility of erythrocytes	Minimum resistance (slight hemolysis): 0.4 to 0.4 per cent sodium chloride		
	Maximum resistance (complete hemolysis): 0.3 t 0.36 per cent sodium chloride		
Leukocytes	Infants: 10,000 to 22,500 per cu. mm.		
	Children: 8,300 to 10,800 per cu. mm.		
	Adults: 6,000 to 10,000 per cu. mm.		
		Adults (Per Cent)	*Children* (Per Cent
Percentages of leukocytes	Nonsegmented neutrophils (metamyelocytes)	2 to 5	3 to
	Segmented neutrophils (mature neutrophils)	54 to 60	16 to 60
	Eosinophils	1 to 3	1 to 3
	Basophils	0.25 to 0.5	0.25 to 0
	Lymphocytes	25 to 33	42 to 4
	Monocytes (large mononuclear cells)	3 to 7	3 to

TABLE 2: SUMMARY OF NORMAL BLOOD VALUES *(Continued)*

Platelets	250,000 per cu. mm. Figures vary with different technics; for example, Dameshek's method[15] gives 500,000 to 900,000 per cu. mm.
Bleeding time	One to three minutes (Duke)
Coagulation time	Capillary tube: one to seven minutes Howell method: ten to thirty minutes Lee and White method: five to eight minutes
Clot retraction time	Begins in one hour; marked in eighteen hours
Prothrombin time	Ten to twenty seconds (Quick[16]); 12.5 per cent plasma, thirty-seven to forty-two seconds; at birth, forty-three seconds
Prothrombin level	300 units per cc. of plasma
Prothrombin index	70 to 100 per cent
Capillary fragility	Suction cup: −20 to −35 cm. of mercury

ERYTHROCYTES

Life Cycle of Erythrocytes:

The normal erythrocyte is a non-nucleated cell that originates in the bone marrow from a nucleated cell called an "erythroblast." Although the nuclei are normally not present in the circulating blood, a few cells retain a blue reticulum when stained with brilliant cresyl blue. These are known as "reticulocytes," and are found in normal blood in values varying from 0.5 to 1.5 per cent.

Opinions differ on the life span of erythrocytes. Dekkers[17] has estimated it as being from fifty to seventy-five days, while Ashby[18] believes they function for from thirty to one hundred days. Vischer[19] has calculated that transfused erythrocytes function only from twelve to thirteen days. The chief function of erythrocytes is to carry oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

Apparently the nonfunctioning cells are removed by the phagocytic cells of the reticuloendothelial system after they have undergone fragmentation. The phagocytic cells of the liver, spleen, bone marrow, and subcutaneous tissues carry on this process. The hemoglobin of the erythrocytes is probably broken up into globin and an iron-containing pigment, hemochromogen in its reduced form and hematin when oxidized. This pigment is freed of iron and is changed to bilirubin, which is a normal constituent of the blood plasma. Bilirubin gives plasma its yellowish tinge and is normally present in a range of from 0.1 to 0.25 mg. per 100 cc. (quantitative van den Bergh test). The fate of globin is not known at present but it is assumed that it is converted into amino acids and used again in manufacturing hemoglobin.

Normal Erythrocytes:

The normal erythrocyte is a round, non-nucleated cell with a thinner central portion. The normal mean diameter of red cells is reported as from 7.2 to 7.9 microns.[20] The normal mean thickness varies, according to different reports, from 1.84 to 2.14 microns.[20] Normal erythrocyte counts are found under Summary of Normal Blood Values (p. 12).

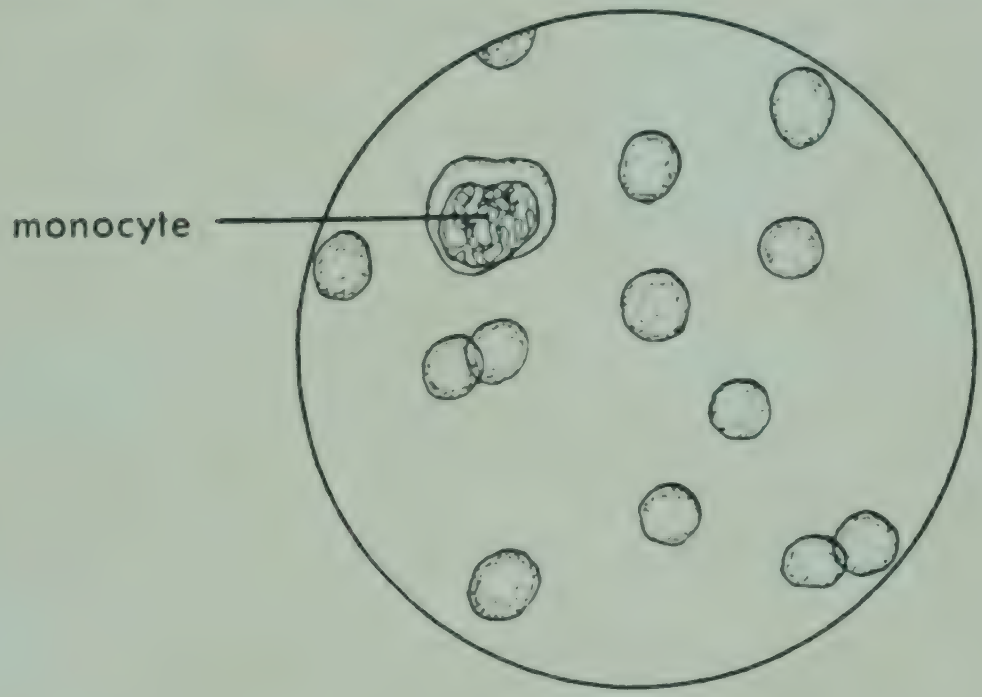
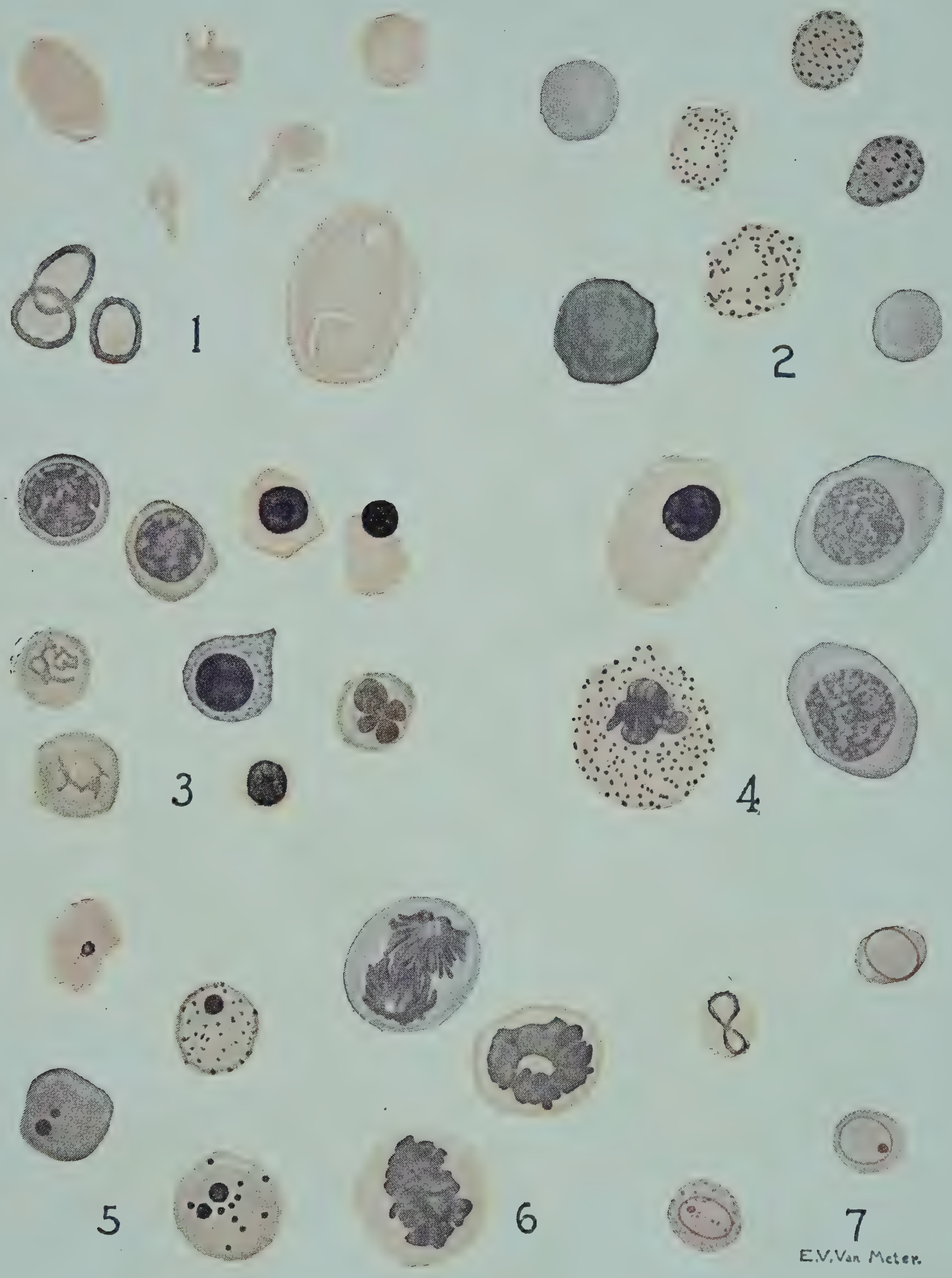


Fig. 1: Normal erythrocytes.

Certain physiological changes take place in the erythrocyte count and hemoglobin value. There is apparently a diurnal variation equivalent to 11 per cent of the mean hemoglobin for the day. Such variation must be correlated with the activity of the person, since there is little or no variation in red-blood-cell count or hemoglobin during complete rest.[21] Muscular activity is usually associated with an increase in red-blood-cell count and hemoglobin value. The ingestion of a large amount of water may temporarily lower the red-blood-cell count, while dehydration will increase this value because of hemoconcentration. Excitement and fear cause an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E.V. Van Meter.

Plate II: Abnormal erythrocytes. (From Todd and Sanford: Clinical Diagnosis by Laboratory Methods. W. B. Saunders Co., Philadelphia.)

increase in the number of red blood cells, which is higher than would be expected from technical variations. Adult males have higher counts than adult females. Low barometric pressure, which is experienced at high altitudes, causes anoxemia and an associated increase in both red blood cells and hemoglobin.

Abnormal Erythrocytes (Plate II):

A differential count should not be considered complete unless a report is made concerning the presence of any abnormal erythrocytes. Certain findings will help the clinician in making a diagnosis.

Certain erythrocytes stain a light blue, polychromatophilia, which is seen in unripe macrocytes or in degenerated nucleated red cells. Variation in size, anisocytosis, is seen in both primary and secondary anemia. Variation in shape, poikylocytosis, is seen in more advanced anemias.

A deficiency of hemoglobin is called "hypochromia." It may be so marked that only a rim is left. These forms occur in hypochromic anemia, sickle-cell anemia, and erythroblastic anemia. Red blood cells may also contain an excess of hemoglobin, hyperchromia. These cells are seen characteristically in macrocytic anemia, such as pernicious anemia.

Basophilic stippling is seen in certain cells. The action of toxins on regenerating red blood cells results in the formation of residual chromatin dots, which take the basic stain. These forms are present in lead poisoning, malaria, and in marked primary and secondary anemias.

Nuclear remnants are sometimes seen. They may appear as blue dots; chromatin particles; spherical, eccentrically located, dark-staining granules; Howell's bodies; and blue-staining, threadlike, twisted rings, Cabot's ring bodies.

Confusing bodies are seen in degenerating red cells: (1) a round body, Maragliano body, has the appearance of a vacuole, and is in the center of the cells; (2) bacillary degeneration, rodlike hyaline areas with a vibratory motion; and (3) Ehrlich's hemoglobinemic degeneration, dark bodies in the centers of red blood cells.

A peculiar cell, called a "target" corpuscle, may be found in hypochromic anemia, sickle-cell anemia, Cooley's anemia, and in jaundice without anemia. This cell is characterized by a central round region of pigmented material, surrounded by a clear zone without pigment, and this in turn is surrounded by the outer border of the cell.

Erythrocytes that take the shape of a sickle when deprived of oxygen are found in sickle-cell anemia, which is peculiar to the negro race. These are not to be confused with elliptical cells, which may be seen normally in both whites and negroes.

Decrease in Number of Erythrocytes:

Erythrocytes are decreased in various forms of anemia (see pp. 325-339).

Increase in Number of Erythrocytes:

"Polycythemia" (see p. 365) is the term used to indicate an increase in the number of erythrocytes in circulating blood. There is also an associated increase in hemoglobin and the volume of packed red blood cells. Erythremia, also known as polycythemia vera, polycythemia rubra, Vaquez's disease, or Osler's disease, is a syndrome of unknown cause in which the red cells are increased to from 7,000,000 to 10,000,000 per cubic millimeter.

A pseudopolycythemia (see p. 366) is seen in the dehydration and hemoconcentration found in shock, vomiting, diarrhea, and severe burns.

The red blood cells are also increased in persons living at high altitudes, in chronic pulmonary disease, in congenital heart disease, and in chronic carbon-monoxide poisoning.

Reticulocytes:

Reticulocytes are seen in the blood smear only when the blood is stained with a vital stain, such as brilliant cresyl blue. They are characterized by a faint blue reticulum in erythrocytic cells that are larger than normal. The normal range is from 0.5 to 1.5 per cent. Newborn infants have from 2 to 6 per cent, but this drops to from 0.5 to 1.5 per cent in from two to five days.

An increased reticulocyte count is indicative of increased erythropoiesis, and is a helpful guide in proving the adequacy of liver therapy in primary macrocytic anemia. The reticulocyte response is in inverse ratio to the original erythrocyte count; that is, the lower the original erythrocyte count, the higher the reticulocyte response.

Reticulocytes are also increased in congenital hemolytic jaundice, sickle-cell anemia, in cases of chronic blood loss of short duration (from two to four months), and in anemic premature infants. There is a slight increase in leukemia, myelophthisic anemia, lead and mercury poisoning, malaria, and in the full-term infant at birth.

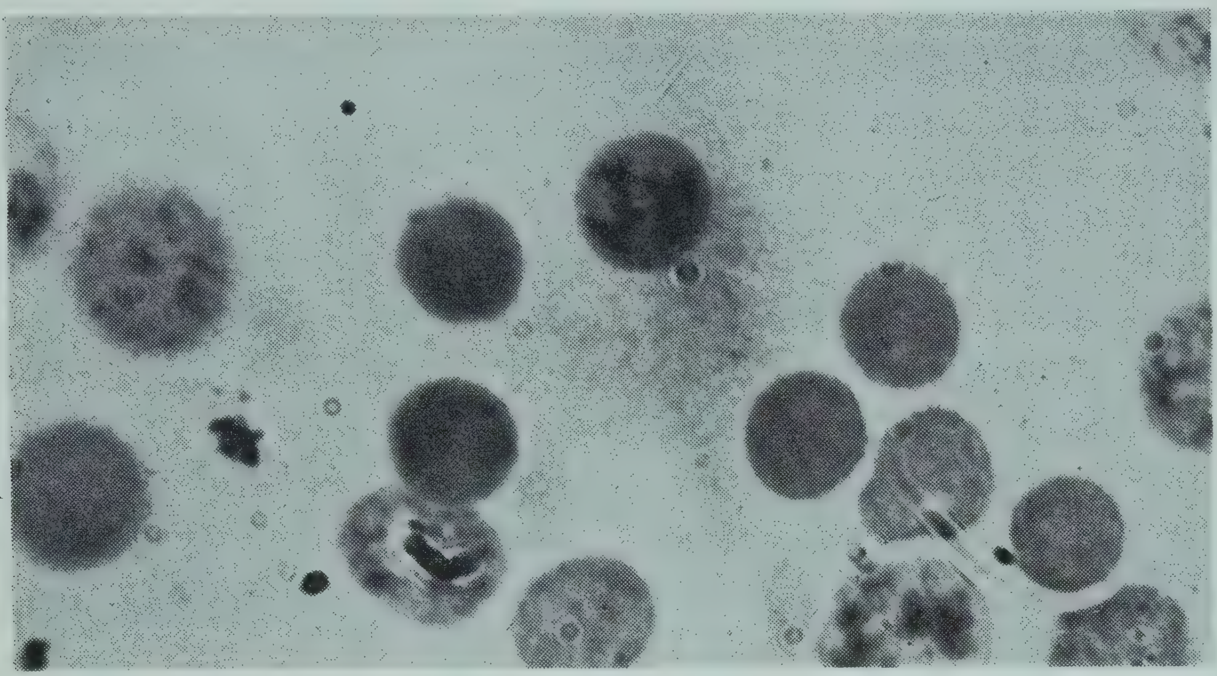

Fig. 2: Reticulocytes.

There is a decrease below low normal (0.5 per cent) in both idiopathic and symptomatic aplastic anemia.

Stippled Cells:

Basophilic stippling is not seen in a smear of normal blood. It does occur in severe anemia, lead and mercury poisoning, leukemia, and chronic malaria. The granules of stippled cells are stained by Wright's stain and are characteristically deep blue. They are not to be confused with the reddish granules seen in erythrocytes containing Plasmodium vivax.

Indices Derived from Erythrocyte Values:

The hematocrit or volume of packed cells in terms of cubic centimeters per 100 cc. is of value in determining alteration in size of erythrocytes. The normal values for cell volume are as follows: infants; 49 to 54 (±10); children from one to fifteen years, 35 to 39; adult males, 47 (±7); and adult females, 42 (±5). It gives clinical information similar to that given by the red-blood-cell count and hemoglobin.

Mean Corpuscular Volume: The mean corpuscular volume may be determined from the volume of packed cells and the erythrocyte count as follows:

$$\frac{\text{volume of packed blood cells, cc. per 100 cc.}}{\text{red blood cell count, millions per cu. mm.}} = \text{number of cubic microns}$$

The value for infants is approximately 84; children, 72; adult males, 82; and adult females, 86 cu. microns. The values are increased to more than 100 in macrocytic anemia and are decreased in microcytic anemia.

Volume Index: The volume index may also be determined by a slightly different formula:

$$\frac{\text{volume of packed blood cells, cc. per 100 cc.} \times 2.3}{\text{red blood cell count, millions per cu. mm.} \times 20}$$

The volume index is the relative mass of circulating red blood cells as compared to the normal. The values for children are from 0.63 to 0.82 and for adults from 0.8 to 1. In macrocytic anemias, values from 1.20 to 1.60 or higher are found.

Sedimentation Rate:

The sedimentation rate of erythrocytes is a nonspecific test, which is accelerated in a variety of diseases and in pregnancy. The reason for this change in rate of fall is not clearly understood. It provides, however, a means of following certain chronic diseases, such as pulmonary tuberculosis and rheumatic fever, provided no other infection is present to influence the test. In cases of anemia, it is advisable to make a correction for the increased rate of fall due essentially to this condition. The corrected figure should then be due to any infection that may be present. In following the progress of a disease, a decrease to normal is favorable, while persistent increased results are not favorable.

The normal results vary with the method used, as indicated in Table 4.

The sedimentation rate remains within normal range in functional nervous disease, benign tumors such as uncomplicated fibroids

TABLE 4: VARIATIONS OF NORMAL SEDIMENTATION RATES

	Cutler (Mm. in One Hour)	*Westergren* (Mm. in One Hour)	*Wintrobe* (Mm. in One Hour)
Children	——	0 to 10	3 to 13.0
Men	0 to 8	0 to 15	0 to 6.5 (average 3.7)
Women	0 to 10	0 to 20	0 to 15.0 (average 9.6)

and cysts, asthma, hay fever, essential hypertension, diabetes mellitus, cirrhosis of the liver, simple peptic ulcer, hydronephrosis, uncomplicated cholelithiasis and nephrolithiasis, early acute appendicitis, head colds, and hypertrophic arthritis.

Changes in the sedimentation rate are shown in Table 5.

Fragility Test:

The fragility test is a measure of resistance of erythrocytes to hypotonic sodium chloride solutions. When normal venous whole blood is used, hemolysis just begins at 0.40 to 0.46 per cent sodium chloride; this is called "minimum resistance." Hemolysis with such blood is usually complete in solutions varying from 0.30 to 0.36 per cent sodium chloride; this is called "maximum resistance."

It is sometimes necessary to employ a modification of the standard fragility test by determining the resistance of erythrocytes to fifth-normal hydrochloric acid, cold, lysolecithin, or saponin. One or more of these modifications may be helpful in demonstrating increased fragility in certain cases of hemolytic anemia.

Increased Resistance: This is found in erythroblastic anemia, pernicious anemia, sickle-cell anemia, hypochromic anemia, hemolytic anemias, erythremia, obstructive jaundice, and after splenectomy.

Decreased Resistance (Increased Fragility): Decreased resistance is marked in congenital hemolytic jaundice (familial hemolytic jaundice). This test helps to differentiate this disease from various types of obstructive jaundice. There is some decrease of resistance in aplastic anemia. This may help to differentiate it from pernicious anemia and hypochromic anemia.

TABLE 5: CHANGES IN SEDIMENTATION RATE

Increased	*Decreased*
Physiological:	Malaria during paroxysms
Pregnancy after second month	Erythremia
Menstruation	Sickle-cell anemia
Infectious processes:	Certain cases of severe liver disease
Acute general and localized infection	
Chronic active infections	
Tuberculosis	
Syphilis	
Rheumatic carditis	
Blood dyscrasias:	
Leukemias	
Anemias	
Poisoning:	
Lead	
Arsenic	
Alcohol	
Miscellaneous:	
Coronary thrombosis	
Hemorrhage	
Advanced malignancy	
Hyperthyroidism	
Nephritis and nephrosis	
Certain cases of severe liver disease	

HEMOGLOBIN

Hemoglobin Formation and Destruction:

Hemoglobin gives the red color to the blood. The red blood cells are, for the most part, made up of water (64 per cent), and the remainder of hemoglobin. Hemoglobin is composed of globin, which is a sulfur-bearing portion (96 per cent), and hematin, a ferrous complex of protoporphyrin (4 per cent). Hemoglobin is formed in the bone marrow in the maturing red blood cells, but the detailed steps are not known.

In the lungs, hemoglobin combines readily with oxygen, forming a compound known as "oxyhemoglobin," which gives arterial blood its bright-red color. The oxygen is given up readily in the tissues, since it is only in loose combination with hemoglobin. Hemoglobin then becomes reduced hemoglobin and carries carbon dioxide from the tissues to the lungs, which accounts for the dark color of venous blood.

After erythrocytes have lived their normal span of life, they undergo fragmentation. Hemoglobin is released and destroyed by the cells of the reticuloendothelial system of the liver (Kupffer's cells), spleen, and bone marrow. The destruction of hemoglobin consists first of separating globin from hematin. Globin is broken down into amino acids. Hematin is broken up by separating the iron, which is stored in the liver and spleen to be used again in the manufacture of hemoglobin. The remaining iron-free product, porphyrin, is converted to bilirubin and transported to the liver to be excreted in the bile. A small amount of bilirubin is excreted in the urine by the kidneys.

Normal Hemoglobin:

In the past, the level of hemoglobin has been expressed in percentage, but this is unsatisfactory because of the variation in different sexes and at different ages, which would require the establishment of arbitrary normals to insure accuracy. In expressing hemoglobin in percentage, it is assumed that 5,000,000 red blood cells per cubic millimeter of blood contain 100 per cent hemoglobin. Hemoglobin is preferably expressed in grams per 100 cc. of blood, using normal values that are corrected for age and sex.

The values given by Cameron and Nicholson[22] for hemoglobin at different ages are shown in Table 6.

Abnormal Hemoglobin Values:

Hemoglobin is increased (hyperchromemia) in those conditions in which there is an increase in erythrocytes. These are erythremia (see p. 365), compensatory polycythemia at high altitudes, and dehydration such as is associated with burns and diarrhea. Hemoglobin is increased in macrocytic anemia (see p. 345) because each erythrocyte carries more hemoglobin than normal (hyperchromia), although the erythrocyte count is lower than normal.

Hemoglobin values are reduced (oligochromemia) in all anemias.

TABLE 6: NORMAL VALUES FOR HEMOGLOBIN

	Hemoglobin (Grams per 100 Cc. Blood)		*Hemoglobin* (in Per Cent) (100 Per Cent = 16.9 Gm. per 100 Cc.)	
Age	Males	Females	Males	Females
At birth	23.2	23.2	137	137
Two days	22.7	22.7	134	134
Four days	22.4	22.4	133	133
Six days	22.1	22.1	131	131
Ten days	21.4	21.4	127	127
Twenty days	20.1	20.1	119	119
One month	19.0	19.0	112	112
Two months	16.2	16.2	96	96
Six months	13.4	13.4	79	79
Nine months	12.9	12.9	76	76
One year	12.6	12.6	75	75
One and one-half years	12.4	12.4	73	73
Two years	12.6	12.6	75	75
Three years	13.1	13.1	78	78
Four years	13.5	13.5	80	80
Five years	13.7	13.7	81	81
Seven and one-half years	14.1	14.1	83	83
Ten years	14.4	14.4	85	85
Twelve and one-half years	14.7	14.7	87	87
Fifteen years	15.4	15.0	91	88
Seventeen and one-half years	16.5	15.3	98	91
Twenty years	16.8	15.4	99	91
Thirty years	16.9	15.4	100	91
Forty years	16.9	15.4	100	91
Forty-five years	16.9	15.4	100	91
Fifty years	16.9	15.9	98	94
Seventy years	15.9	15.4	94	91
Eighty years	15.7	15.1	93	89

In hypochromic anemias particularly, the hemoglobin is low, not only because there is a reduction in total erythrocytes, but also because the individual cells have a decreased amount of hemoglobin.

Indices Derived from Hemoglobin Values:

Color Index: In computing the color index, it is assumed that 5,000,000 red blood cells per cubic millimeter of blood have a hemoglobin value of 100 per cent. The color index is determined by dividing the percentage of hemoglobin by the first two figures of the erythrocyte count multiplied by 2. A hemoglobin value of 70 per cent with a red-blood-cell count of 4,000,000 would give a color index of $\frac{70}{80}$, or 0.87, which would suggest hypochromic anemia. A color index greater than 1 is indicative of macrocytic anemia.

Mean Corpuscular Hemoglobin: This index is computed by dividing the hemoglobin value in grams per 1000 cc., by the erythrocyte level, in millions per cubic millimeter, and expressing the results in micromicrograms. The normal for adults is 29 (± 2); for children, from 26 to 28.

Mean Corpuscular Hemoglobin Concentration: This index is calculated by dividing hemoglobin, in grams per 100 cc., by the volume of packed cells, in cubic centimeters per 100 cc. of blood, and multiplying by 100. The results are given in grams per 100 cc. The normal for adults is 34 (± 2); for children, from 32 to 34. The mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration are decreased in hypochromic anemias and increased in macrocytic anemias.

Saturation Index: The saturation index is computed as follows:

$$\text{Saturation index} = \frac{\text{hemoglobin, per cent (gm. per 100 cc.} \times 6.9)}{\text{volume packed red blood cells (cc. per 100 cc.} \times 2.3)}$$

The saturation index is decreased in hypochromic and microcytic anemias, and increased in macrocytic anemias.

LEUKOCYTES

Types of Leukocytes:

Leukocytes of the peripheral blood are divided into two general groups, the granulocytes and the nongranulocytes. The granulocytes consist of polymorphonuclear neutrophils, eosinophils, and basophils. The nongranulocytes consist of lymphocytes and monocytes (large mononuclear cells).

Origin and Significance of Granulocytes:

Polymorphonuclear Neutrophils: The youngest recognized forms of this series are the myeloblasts. Normally, these cells occur only in the bone marrow, where they go through certain stages to reach maturity. Myeloblasts are difficult to differentiate from lymphoblasts and monoblasts. The myeloblast next develops into the myelocyte, which is characterized by the presence of tiny, pale, red-staining granules in the cytoplasm. Myeloblasts and myelocytes are not found in the peripheral blood except in conditions that stimulate great activity in the bone marrow, such as severe infections, myelogenous leukemia (see p. 368), and erythroblastosis foetalis (see p. 335).

METAMYELOCYTES: Normal values are as follows: adults, from 2 to 5 per cent, children, from three to ten years old, from 3 to 8 per cent. The myelocyte becomes a metamyelocyte by the indentation of the nucleus and later assumes the shape of a horseshoe. The fine, pale-red granules remain in the cytoplasm. Metamyelocytes may be divided into young forms (with nucleoli and dark-staining nuclei) and adult forms (without nucleoli). Metamyelocytes are the youngest forms that are found normally in the peripheral blood. They are increased in acute infectious processes, with or without an increase in the total leukocyte count. An increase in metamyelocytes constitutes a shift to the left, as described by Schilling.

SEGMENTED POLYMORPHONUCLEAR NEUTROPHILS: Normal values are as follows: adults, from 54 to 60 per cent; children, from three to ten years old, from 16 to 60 per cent. The nucleus of the metamyelocyte becomes pinched off into from two to six or even ten lobes connected by threads of chromatin. Normally, the granules remain pale red, but in severe infections they may take the basophilic stain and increase in size. These are toxic granules, which are thought to be due to the effect of toxins in the cells.

Segmented neutrophils are increased in acute infections, myelogenous leukemia, coronary thrombosis, and intestinal obstruction. The percentage of adult neutrophils is decreased in agranulocytosis, benzene poisoning, arsenic poisoning, typhoid and paratyphoid fevers, malaria, leishmaniasis, undulant fever, some cases of severe infection, infectious hepatitis, pernicious anemia, hypochromic anemia, and after treatment with barbiturates, aminopyrine, sulfonamides, thiouracil, and intense radiation by x-ray.

Eosinophils: Normal values are as follows: adults and children, from 1 to 3 per cent. Eosinophils originate from myeloblasts; when they develop into myelocytes, however, large acid-staining granules form in the cytoplasm. The adult cells rarely have more than two lobes, but they do retain the coarse, acid-staining granules.

Eosinophils are increased in trichinosis, hydatid cyst, hookworm infection, strongyloidiasis, asthma, anaphylaxis, myelogenous leukemia, psoriasis, eczema, pemphigus, scarlet fever, and sometimes malignant granuloma. They are increased also in streptomycin therapy and range from 8 to 12 per cent. Both young and adult forms are increased in eosinophilic leukemia. They are decreased in infectious conditions, including pneumonia.

Basophils: Normal values are as follows: adults and children, from 0.25 to 0.5 per cent. Basophils originate from myeloblasts, but the mature nuclei do not become lobulated. The cytoplasm is characterized by coarse, basic-staining granules. These cells are increased in myelogenous leukemia, basophilic leukemia, pernicious anemia, and erythremia.

Origin and Significance of Nongranulocytes:

Lymphocytes: Normal values are as follows: adults, from 25 to 30 per cent; children, from three to ten years old, from 42 to 48 per cent. Lymphocytes supposedly originate from lymphoblasts in the spleen, lymph glands, tonsils, thymus, and possibly the bone marrow. Lymphoblasts are not present normally in the peripheral blood, but they are found in blood smears of acute lymphatic leukemia.

Lymphocytes are sometimes classified as "large" and "small," the large form representing the more immature stage. Some authorities assert, however, that the large forms originate in the spleen, while the small forms originate in the lymph glands.

Lymphocytes are increased in lymphatic leukemia, infectious mononucleosis, tuberculosis, syphilis, rickets, pertussis, and parotitis. An increased lymphocyte percentage with a low total white-blood-cell count is found in typhoid fever, pernicious anemia, influenza, undulant fever, and familial splenic anemia (Gaucher's disease).

Monocytes, or Large Mononuclear Cells: Normal values are as follows: adults, from 3 to 7 per cent; children, from three to ten years old, from 3 to 5 per cent. Monocytes are believed to originate in the bone marrow from monoblasts. These cells develop into premonocytes, which in turn become adult monocytes.

Monocytes are increased in infectious mononucleosis, monocytic leukemia, tuberculosis, subacute bacterial endocarditis, undulant fever, typhus fever, malaria, systemic leishmaniasis, Rocky Mountain spotted fever, tetrachlorethane poisoning, malignant granuloma, and familial splenic anemia (Gaucher's disease).

Plasma Cells:

Plasma cells are thought to originate from lymphocytes or primitive, connective-tissue cells.[23] They are round cells with deep blue cytoplasm and an eccentric round nucleus containing deep-staining chromatin arranged like the spokes of a wheel. Plasma cells are not considered in the normal differential count because they are rarely found in the blood smear. They are seen in sections of tissue representing chronic inflammation.

Normal Total Leukocyte Values:

The total leukocyte count varies normally with age, exercise, relationship to a meal, sunlight, high altitudes, and dehydration. There may also be physiological changes in the leukocyte count from day to day. Normal values for various age groups are as follows:

	Per Cubic Millimeter
Infants	10,000 to 22,500
Children	8,300 to 10,800
Adults	6,000 to 10,000

Leukocytosis:

"Leukocytosis" is the term applied to an increase in the total leukocyte count above the upper limit of normal. Leukocytosis is caused by the absolute increase in number of one of the white cells; thus, an increase in neutrophils (neutrophilia) in acute appendicitis or of eosinophils (eosinophilia) in trichinosis will cause an increase in the total white count.

Causes of Leukocytosis: The important causes of leukocytosis may be grouped as follows:

PHYSIOLOGICAL CONDITIONS: Strenuous exercise, pregnancy, labor, puerperium, during digestion, after a hot bath, fear, pain, dehydration, sunlight, and high altitude.

ACUTE AND CHRONIC INFECTIONS: Infections caused by certain bacteria and fungi, septicemia, osteomyelitis, salpingitis, smallpox, scarlet fever, gonorrhea, rheumatic fever, tetanus, pertussis, anthrax, cholera, plague, pneumonia, pemphigus, tonsillitis, otitis media, pyelitis, carbuncle, ulcer, and coccidioidal granuloma.

METABOLIC DISEASES: Uremia, diabetic acidosis, and eclampsia.

POISONS AND DRUGS: Mercury, lead, digitalis, epinephrine, venoms, and foreign proteins.

PARASITIC DISEASES: Trichinosis, strongyloidiasis, and ancylostomiasis.

DISEASES OF THE HEMOPOIETIC SYSTEM AND BLOOD: Myelogenous leukemia, eosinophilic leukemia, basophilic leukemia, lymphocytic leukemia, monocytic leukemia, lymphosarcoma, malignant granuloma, sometimes erythremia, sickle-cell anemia, erythroblastosis foetalis, acute hemorrhage, and incompatible transfusions.

MISCELLANEOUS: Rapidly growing tumors, periarteritis nodosa, infectious mononucleosis, and after prolonged vomiting.

Leukopenia:

Leukopenia is a reduction in the total number of leukocytes below the lower limit of normal. Certain physiological states, diseases, poisons, and drugs cause a decrease in the total leukocyte count.

Causes of Leukopenia: The important causes of leukopenia may be grouped as follows:

PHYSIOLOGICAL CONDITIONS: Cachexia and debilitated states.

ACUTE AND CHRONIC INFECTIONS: Infections caused by certain bacteria, viruses, and protozoa, typhoid fever, paratyphoid fever, undulant fever, influenza, measles, rubella, miliary tuberculosis, overwhelming septicemia, malaria, relapsing fever, and leishmaniasis.

DISEASES OF THE HEMOPOIETIC SYSTEM: Aplastic anemia, agranulocytosis, aleukemic leukemia, relapse of pernicious anemia, chronic hypochromic anemia, Banti's disease, and familial splenic anemia (Gaucher's disease).

DRUGS AND POISONS: Sulfonamides, barbiturates, aminopyrine, and benzene.

RADIATION: X-ray, radium, and radiation from atomic disintegration.

MISCELLANEOUS: Portal cirrhosis, catarrhal jaundice, anaphylactoid shock, nonspecific-protein therapy, and rheumatoid arthritis

PLATELETS

Life Cycle of Platelets:

The platelets are thought to originate from the megakaryocytes of the bone marrow. The mechanism of their formation is not clear, but apparently the megakaryocytes undergo fragmentation to form platelets. They must have a short span of life because all of the platelets in the peripheral blood can be replaced in from three to five days.

Platelets are concerned in the clotting of blood and in clot retraction, but just how they function is not known. When platelets are reduced in number, blood clots are less adhesive, retract less readily, and are less firm.

Normal Platelet Values:

The counting of platelets is not satisfactory because of their small size, agglutination potential, and tendency to break up. The usual range is 250,000 to 500,000 per cubic millimeter.

Thrombocytosis:

An increase above the upper limit of normal is called "thrombocytosis."

Causes of Thrombocytosis: Thrombocytosis is noted in the following:

PHYSIOLOGICAL CONDITIONS: High altitudes, severe exercise, cachexia, and malnutrition.

DISEASES OF HEMOPOIETIC SYSTEM AND BLOOD: Erythremia, hemolytic anemias, anemia following hemorrhage, chronic myelogenous leukemia, and chlorosis.

INFECTIONS: Suppurative processes and acute rheumatic fever.

MISCELLANEOUS: Fractures of bones and asphyxia.

Thrombocytopenia:

A decrease in the total platelet count (see pp. 361-363) below the lower limit of normal is called "thrombocytopenia." This condition is found mostly in primary and secondary purpura hemorrhagica as follows:

1. Idiopathic thrombocytopenic purpura
2. Symptomatic thrombocytopenic purpura caused by the following: chemical and physical agents; acute and chronic leukemias; hypochromic, aplastic, pernicious, and myelophthisic anemias; certain infections, such as septicemia, typhoid fever, early pneumonia, diphtheria, and bacterial endocarditis; hemolytic jaundice; Banti's disease; familial splenic anemia (Gaucher's disease); and menstruation on the first day

COAGULATION OF BLOOD

The nature of the mechanism involved in the coagulation of blood is disputed. For practical purposes, however, it is made comparatively simple by Eagle,[24] who proposes a combination of the Morawitz and Schmidt theories to make two successive enzyme reactions. According to this theory, the platelets act in conjunction with calcium to form an enzyme that activates prothrombin; the resulting thrombin acts as an enzyme that activates fibrinogen to produce fibrin. This is resolved into two steps by Wintrobe[25] (Table 7).

Coagulation Time:

Coagulation time is the measurement of the clotting of blood in vitro without tissue juices. This may be done in several ways, but the same technic and the same caliber glassware should be used each time.

TABLE 7: ENZYME THEORY OF BLOOD COAGULATION

Step	Substrate				
1.	Prothrombin	+	calcium + platelets)	⟶	thrombin
2.	Fibrinogen	+	thrombin	⟶	fibrin

Capillary-Tube Method: A common technic is the capillary-tube method. Five pieces of capillary tubing are filled with fresh blood from a deep cut. A section of tube is broken off at each one-half-minute interval. The end-point is the appearance of a thin thread of fibrin between the fragments as they are pulled apart. The normal coagulation time by this method is from three to seven minutes.

Lee and White Method: Another popular method is that of Lee and White, in which 1 cc. of blood is placed in a tube with a diameter of 8 mm. ($^{3}/_{10}$ inch). The tube should be absolutely clean and should be rinsed with isotonic saline solution. It is advisable to use at least three tubes so that one tube is undisturbed while the others are being examined. The test is best carried out at 98.6° F. (37° C.). The normal range by this method is from six to fifteen minutes.

The coagulation time is prolonged in those diseases in which there is a deficiency of prothrombin, either because a diseased liver is unable to use vitamin K in its production or because of a dietary deficiency of vitamin K or inability to absorb it. It is also prolonged in hemophilia and in liver disease, which prevents the formation of fibrinogen.

Clot-Retraction Time:

Determination: Determination of clot-retraction time may be made by placing 1 cc. of venous blood in each of three small tubes (8 mm. [$^{3}/_{10}$ inch] in diameter) and 3 cc. in a fourth. All tubes are placed in a 98.6° F. (37° C.) incubator and inspected (not handled) after one-half, one, two, four, and twenty-four hours for evidence of retraction from the side. There is retraction of normal blood in from thirty to sixty minutes.

Clot retraction is prolonged and is not satisfactory in thrombocytopenic purpura, aplastic anemia, pernicious anemia, hemorrhagic disease of the newborn, acute leukemias, multiple myeloma, and malignant granuloma (Hodgkin's disease) involving the bone marrow.

Bleeding Time:

Determination: To determine the bleeding time, a cut deep enough to produce a good drop of blood without pressure is made in the ear lobe or finger. The blood is removed every half minute by filter paper without touching the skin. The normal range is from one to three minutes.

The bleeding time is prolonged in thrombocytopenic purpura, pernicious anemia, aplastic anemia, infectious mononucleosis, hemorrhagic disease of the newborn, acute leukemias, chronic lymphatic leukemia, malignant granuloma (Hodgkin's disease) of the bone marrow, and multiple myeloma.

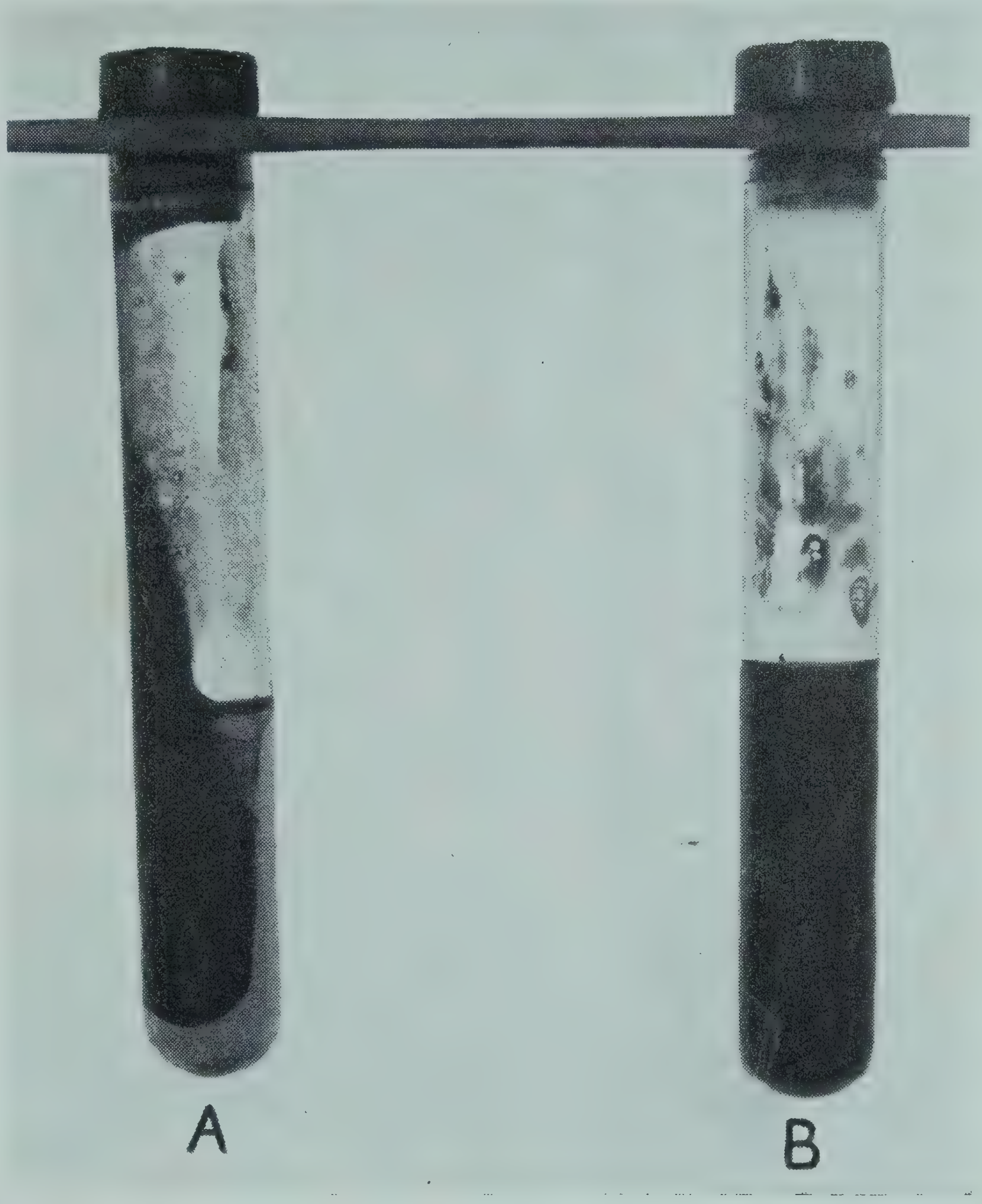

Fig. 3: Clot retraction. *A*: Normal retraction of clot. *B*: No retraction of clot.

Capillary-Fragility Test:

Determination: The capillary-fragility test is also known as the "tourniquet test." A blood-pressure cuff is applied above the elbow, and the pressure is maintained at a point just above the diastolic pressure. In a positive test, a large number of tiny hemorrhages develop within a few minutes.

The test is positive in idiopathic thrombocytopenic purpura and in some cases of symptomatic thrombocytopenic purpura, scurvy, scarlet fever, measles, influenza, achylia gastrica, vitamin-K deficiency, and chronic nephritis.

Prothrombin Determination:

Schofield's discovery of the part played by spoiled sweet clover in hemorrhagic disease of cattle was followed by isolation and synthesis of the active principle, dicumarol. Dicumarol and heparin now have important roles in the prevention and treatment of phlebothrombosis and thrombophlebitis. In such cases, the blood-clotting mechanism is too efficient. The use of dicumarol and heparin is an attempt to reduce the efficiency of this mechanism to the lower limit of safety. This is done by giving an initial dose of from 200 to 300 mg. (3 to 4½ grains) of dicumarol. A prothrombin time or prothrombin activity (Fig. 4) is determined at this time to establish a base line, or normal, for the patient. To eliminate confusion, the prothrombin time only is reported in seconds. The complete report should include the prothrombin time of the control as well as that of the patient. The range of the normal prothrombin time is from ten to twenty seconds, usually from twelve to fourteen seconds.

In view of the fact that a difference of a few seconds in prothrombin time is indicative of wide differences in prothrombin concentration, procedures have been developed in which the plasma is diluted, thus creating a longer coagulation time and making it easier to detect minor variations in prothrombin concentration. The optimal dilution appears to be 12.5 per cent plasma in isotonic saline solution, and this is said to increase the sensitivity and reliability of the test. The normal range using diluted plasma according to the method of Shapiro[31] is from thirty-seven to forty-two seconds.

Sources of Error: The physician as well as the technician should be aware of the sources of error in determining the prothrombin time.

1. Fresh plasma should be used. Plasma allowed to stand at room temperature or ice-box temperature for more than two or three hours may give clotting times slightly higher than fresh plasma.

2. Hemolysis of the specimen of blood.

3. Inadequate amount of blood resulting in an excess of the anticoagulant.

4. The use of old thromboplastin extract. It should be used within four hours after preparation. Prolonged aging results in a loss of potency and prolonged clotting time.

5. Metallic ions, such as copper, in the distilled water used in making the saline solution for extraction of the thromboplastin may lead to prolonged clotting time.

6. Failure to recognize the end-point.

7. Glassware must be chemically clean. Soaps and other detergents used in cleaning glassware, if not properly rinsed off, will tend to give prolonged clotting time.

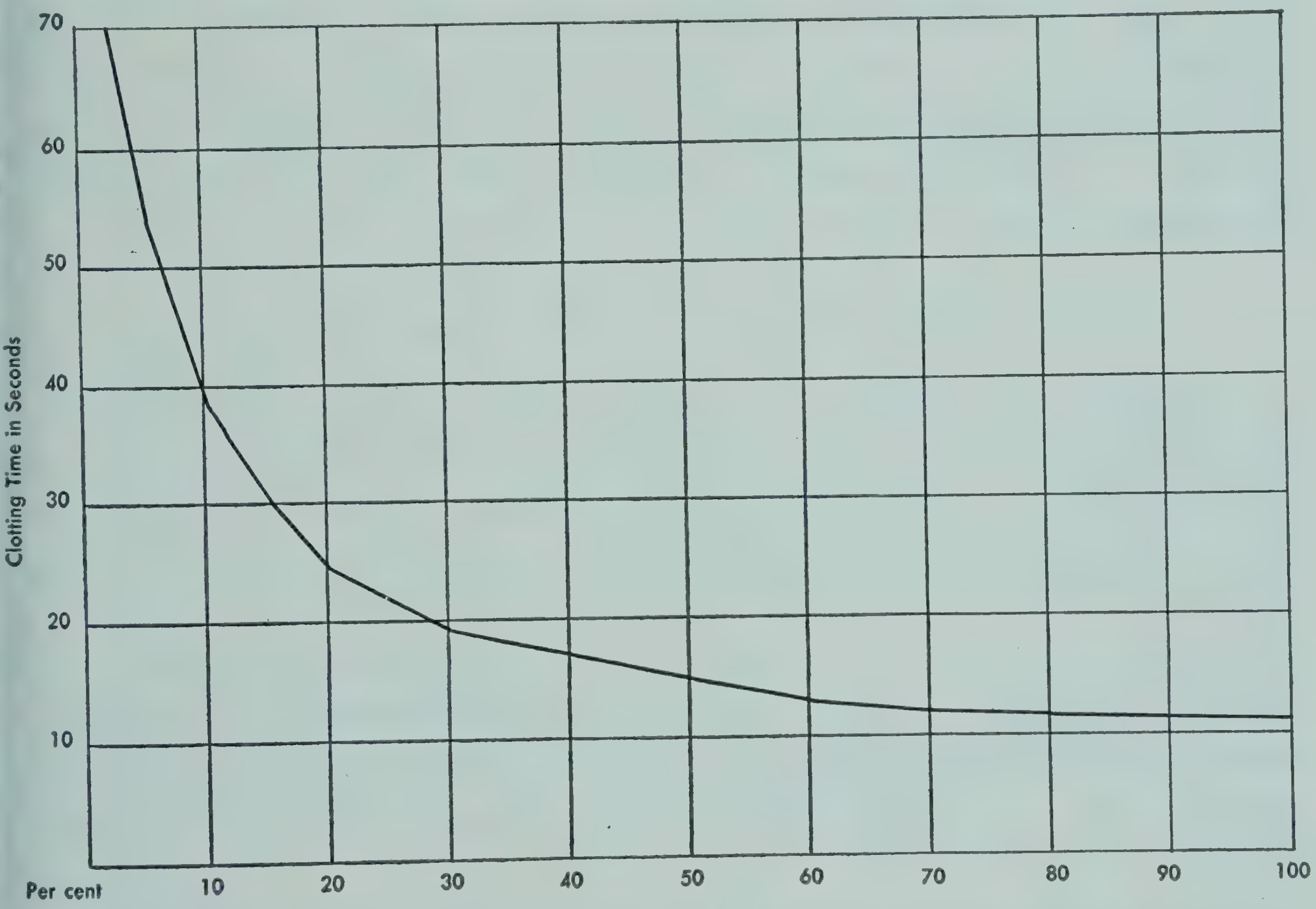

Fig. 4: Prothrombin activity curve for plasma with prothrombin time of twelve seconds. Curve is determined by tests on serial dilutions of plasma. (From Quick, A. J.: Am. J. Clin. Path., 10:222, 1940.)

8. The calcium chloride, sodium chloride, sodium oxalate, potassium oxalate, and ammonium oxalate must be anhydrous and of reagent grade. Prolonged clotting times have been traced frequently to faulty calcium chloride. It should be used in an anhydrous state.

9. Failure to mix the reagents thoroughly with the plasma at the time of addition may result in a prolonged clotting time.

10. The storage of thromboplastin at room temperature may result in slight loss of potency in one to two months. It is, therefore, advisable to store it in the ice box.

11. The use of improper technique, such as improper use of the pipet, neglecting to blow out after each transfer, and neglecting to wipe the outside of the pipet at each transfer.

Formol-Gel Reaction:

The formol-gel reaction is a means of determining the level of hyperglobulinemia or hyperfibrinogenemia. The technic consists in adding two drops of 40 per cent formaldehyde to 1 cc. of serum. A milky gel, or coagulation, develops if the test is positive. The test is positive in rheumatoid arthritis and is an index of the systemic activity of the disease, according to Scull and Pemberton.[28] It may also be positive in multiple myeloma and visceral leishmaniasis (kala-azar).

NEW TERMINOLOGY OF BLOOD CELLS

The first two reports [29,30] of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs recommend certain changes in names and definitions of blood cells. It seems advisable to give a summary of these recommendations here with the hope that it will help to promote a universal adoption of some form of standard nomenclature.

The following terms are recommended for the cells of the different series and for diseases affecting any cell of that series: lymphocytic, granulocytic, monocytic, plasmacytic, thrombocytic, and erythrocytic.

Table 8 gives recommended terms for each of the white cells and thrombocytes.

The definitions decided on are as follows:

Lymphoblast:

Any cell of the lymphocytic series having fine chromatin structure in the nucleus. Cells of blast morphology associated with prolymphocytes should be tentatively classified as lymphoblasts.

Prolymphocyte:

Any cell of the lymphocytic series intermediate in morphology between the lymphoblast and the lymphocyte. It will always have too coarse a chromatin structure to fit the criteria for a blast and too fine a chromatin structure or too large a cell diameter to be classed as a lymphocyte. Usually, but not always, prolymphocytes are larger than 15 microns in diameter, which is the upper limit for the lymphocyte.

Lymphocyte:

Any cell of the lymphocytic series having the morphology of those commonly found in the blood of healthy adults.

Monoblast:

Any cell of the monocytic series having fine chromatin structure. Usually nucleoli are visible. Cells of blast morphology found in association with promonocytes should be tentatively classed as monoblasts.

Promonocyte:

Any cell intermediate in morphology between the monoblast and the monocyte. It is differentiated from the monoblast by having an irregularly shaped nucleus and somewhat coarser chromatin structure, and from the monocyte by the presence of one or more nucleoli.

Monocyte:

Any cell of the monocytic series having the morphology of those commonly found in the blood of healthy adults. It is differentiated from the promonocyte by the absence of nucleoli.

Myeloblast:

Any cell of the granulocytic series having fine chromatin structure and containing no specific granules. Usually nucleoli are visible. Cells of blast morphology found in association with progranulocytes should tentatively be classed as myeloblasts.

Progranulocyte:

Any cell of the granulocytic series which has a nuclear structure too coarse for that of a blast cell and which has not yet developed

TABLE 8: RECOMMENDED TERMS AND TERMS TO BE AVOIDED WHEN REFERRING TO SPECIFIC CELLS OF BLOOD AND BLOOD-FORMING ORGANS

Name of Series	*Term to be Used*	*Terms to be Avoided*
Lymphocytic. .	Lymphoblast	Myeloblast, hemocytoblast, lymphoidocyte, stem cell, lymphocyte
	Prolymphocyte	Large lymphocyte, pathologic large lymphocyte, atypical leukocytoic lymphocyte, monocyte, immature lymphocyte
	Lymphocyte	Small, medium, or large lymphocyte, normal lymphocyte, small, medium, or large mononuclear
Monocytic. . . .	Monoblast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, immature monocyte
	Promonocyte	Premonocyte, hemohistioblast, immature monocyte, Ferrata cell
	Monocyte	Large mononuclear, transitional, plasmatocyte, endothelial leukocyte, histiocyte, resting wandering cell
Granulocytic. .	Myeloblast	Granuloblast, hemocytoblast, lymphoidocyte lymphocyte, stem cell
	Progranulocyte	Promyelocyte II, leukoblast, myeloblast, premyelocyte, promyelocyte, progranulocyte A
	Myelocyte	Granulocyte, myelocyte B, nonfilament, class I

TABLE 8: *(Continued)*

Name of Series	*Term to be Used*	*Terms to be Avoided*
Granulocytic. *Continued*	Metamyelocyte	Metagranulocyte, juvenile, myelocyte C, nonfilament, class I
	Band cell	Staff cell, stab cell, nonfilament, class I, rod nuclear, polymorphonuclear, stabkernige, rhabdocyte, nonsegmented
	Segmented	Polymorphonuclear, filamented, class II, III, IV, or V, lobocyte
Plasmacytic...	Plasmablast	Myeloblast, hemocytoblast, lymphoidocyte, lymphocyte, stem cell, lymphoblastic plasma cell, myeloma cell
	Proplasmacyte	Türk cell, Türk irritation form, lymphoblastic or myeloblastic plasma cell, myeloma cell
	Plasmacyte	Plasma cell, Unna's plasma cell, Marschalko's plasma cell, plasmacytoic lymphocyte, myeloma cell
Thrombocytic.	Megakaryoblast	Megalokaryoblast
	Promegakaryocyte	Premegalokaryocyte
	Megakaryocyte	Megalokaryocyte
	Thrombocyte	Platelet, thromboplastid
	Disintegrated cell	Senile cell, smudge, basket cell, smear cell, degenerated cell

discernible, specific granules. This term was selected rather than "promyelocyte" because of its clear relationship to the definition of granulocyte, given below, and because the term "promyelocyte" has been in wide use for cells which do contain specific granules. The reason that the terms "granuloblast," "granulocyte," and "metagranulocyte" were not chosen was that the terms "myeloblast" and "myelocyte" were already in general use with essentially the definitions here given. This is true also for the term "granulocyte," which would otherwise have to be synonymous with the term "myelocyte."

Specific Granules:

Neutrophilic, eosinophilic, or basophilic granules. This term does not include azurophilic granules.

Granulocyte:

An inclusive term to apply to any cell containing specific granules. The plural form "granulocytes" would therefore include all myelocytes, metamyelocytes, band cells, and segmented cells whether neutrophils, eosinophils, or basophils.

Myelocyte:

Any cell containing specific granules, with a round or oval nucleus. It is distinguished from the progranulocyte by the presence of specific granules and from the metamyelocyte by the absence of indentation in the nucleus. It may be further subdivided, at the option of the user, into early and late stages, but the definition of early or late should be clearly stated in any publication.

This and all subsequent cells of the granulocytic series should be additionally characterized as neutrophil, eosinophil, or basophil.

Metamyelocyte:

Any cell of the granulocytic series having specific granules in the cytoplasm and a nucleus intermediate in shape between that of the myelocyte and the band cell. The nucleus usually has an indented oval shape, resembling a bean or kidney.

Band Cell:

Any cell of the granulocytic series which has a nucleus that could be described as a curved or coiled band, no matter how marked the indentation, if it does not completely segment the nucleus into lobes connected by a filament. It is differentiated from the metamyelocyte by an appreciable length of the nucleus having parallel sides, and from the segmented neutrophil by having no indentation which could be described as a filament.

Segmented Cell:

Any cell containing specific granules in which the lobes of the nucleus are connected by a filament. A filament is defined as a thread-like structure. Since at times, in viewing a three-dimensional object from one direction, it is impossible to be certain whether two parts of the nucleus are connected by a filament or band, it is suggested that such cells always be placed in the segmented category, since this is the more differentiated and more common cell.

The term "toxic neutrophils," followed by a 1 to 4+ designation, is recommended for the grading of toxic granules, basophilia of the cytoplasm, vacuoles, and condensation of nuclear chromatin in the neutrophils, since its meaning is clear, although it is recognized that it is not an adequately descriptive term. The grading should depend more on the degree of change than on the percentage of the cells involved and should be recorded in the report whenever the degree of change exceeds 2+.

Plasmablast:

Any cell of the plasmacytic series having fine chromatin structure in the nucleus. Cells of blast morphology found in association with proplasmacytes are usually seen only in plasmacytic leukemia or plasmacytic sarcoma. The cytoplasm tends to be more opaque in staining than in the other leukocytic blast cells.

Proplasmacyte:

Any cell of the plasmacytic series with a nuclear structure too coarse for that of a blast cell but with one or more nucleoli present.

Plasmacyte:

A cell characterized by extremely coarse chromatin structure, with the deeply staining chromatin of the nucleus aggregated into large, sharply demarcated clumps. It is differentiated from the proplasmacyte by the absence of nucleoli. The cytoplasm of all cells of the plasmacytic series tends to be deeply basophilic and opaque in appearance. Azurophilic granules may be present or absent, but are more commonly absent.

Megakaryoblast:

Any cell of the thrombocytic series having a nucleus with fine chromatin structure. Usually these are larger than the other blast cells.

Promegakaryocyte:

Any cell of the thrombocytic series with a nucleus containing nucleoli but having a chromatin structure too coarse for a blast cell. The nucleus is usually similar in shape to that of the megakaryocyte. Fine azurophilic granules are usually diffusely scattered through the cytoplasm.

Megakaryocyte:

Any nucleated cell of the thrombocytic series in which nucleoli are not discernible. The azurophilic granules are often aggregated into clumps. Megakaryocytes and promegakaryocytes are typically much larger than other cells found in the marrow.

Thrombocyte:

Any cell of the thrombocytic series containing no nucleus; in other words, any nonnucleated fragment of megakaryocytic cytoplasm containing azurophilic granules similar to those of the mature megakaryocyte.

The term "thromboplastid" was recognized as being anatomically correct, but it was felt that to be consistent with the use of the term "erythrocyte" and to permit the use of "thrombocytic" and "erythrocytic" in describing these cell series, the suffix "cyte" was preferable for these two nonnucleated forms.

Disintegrated Cell:

Any cell of any series in which the cytoplasmic outline has been disrupted or the nuclear chromatin is no longer surrounded by a membrane, excluding the changes in the nucleus that occur in mitotic division. Disintegrated cells should be recorded as such in the differential report, even though they could be identified by dispersed granules. They should be counted even if only shreds of nuclear material are discernible, since they are undoubtedly included in the total leukocyte count.

Table 9 gives the recommended terms for cells of the erythrocytic series.

The definitions of cells of the erythrocytic series are as follows:

Rubriblast:

Any cell of the erythrocytic series having fine chromatin structure in the nucleus. Nucleoli are usually discernible. A stippled chromatin pattern is more common than the lace-net pattern usually seen in other blast cells.

TABLE 9: RECOMMENDED TERMS AND TERMS TO BE AVOIDED WHEN REFERRING TO SPECIFIC CELLS OF ERYTHROCYTIC SERIES

Name of Series	*Term to be Used*	*Terms to be Avoided*
Erythrocytic . .	Rubriblast	Erythroblast, megaloblast, pronormoblast, promegaloblast, normoblast, hemocytoblast, stem cell, myeloblast, lymphoidocyte, karyoblast
	Prorubricyte	Erythroblast, megaloblast, pronormoblast, normoblast, macronormoblast, macroblast, prokaryocyte
	Rubricyte	Normoblast, pronormoblast, macronormoblast, erythroblast, polychromatophilic normoblast, karyocyte
	Metarubricyte	Normoblast, erythroblast, metakaryocyte
	Reticulocyte*	
	Erythrocyte	Red blood cell, erythroplastid, normocyte, akaryocyte

* It is recommended that the reticulocyte stage be considered a subdivision of the erythrocyte stage.

Prorubricyte:

Any cell of the erythrocytic series in which one or more nucleoli are discernible in the nucleus and which has a chromatin structure too coarse to be classified as a rubriblast.

Rubricyte:

Any cell of the erythrocytic series having definite structure of the nuclear chromatin, but containing no discernible nucleoli. This stage is differentiated from the prorubricyte by the absence of nucleoli in the nucleus, and from the metarubricyte by not having a pyknotic, fragmented, or partially extruded nucleus. Some may wish to subdivide and qualify this stage—or other stages—further into basophilic, polychromatic, or normochromatic rubricytes, according to the amount of hemoglobin present in the cytoplasm.

Metarubricyte:

Any nucleated cell of the erythrocytic series having a pyknotic, fragmented, partially extruded, or partially autolyzed nucleus. Pyknotic describes a dense, solid, structureless nuclear mass. The phenomenon of karyorrhexis, or fragmentation of nuclei, should be clearly distinguished from the occurrence of double, well-formed nuclei, which are occasionally seen in prorubricytes and rubricytes, as well as in other cells which may divide amitotically.

Reticulocyte:

Any nonnucleated cell of the erythrocytic series in which, when supravitally stained—usually with brilliant cresyl blue—one or more granules or a diffuse network of fibrils are discernible. All reticulocytes are included under the term "erythrocytes" since, without a special stain, reticulocytes are indistinguishable from erythrocytes.

Erythrocyte:

Any nonnucleated cell of the erythrocytic series.

Pernicious-Anemia Type:

The qualifying adjective phrase to be applied to any cell of the erythrocytic or granulocytic series, and to the marrow and blood pictures as a whole, to indicate the presence of the morphologic changes characteristically seen in pernicious anemia and other macrocytic anemias which respond to liver extract or folic-acid therapy. In the nucleated cells of the erythrocytic series, the major feature of this change is a relative increase in the pale-staining parachromatin with a corresponding decrease in the deep-staining basichromatin. In the cells of the granulocytic series, the characteristic change is the presence of giant forms having very bizarre nuclei; and in the segmented neutrophils, the occurrence of many cells with more than five lobes.

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CHAPTER 2

Blood Chemistry

Introduction:
The blood contains a variety of organic and inorganic constituents, the determination of which is an aid in diagnosis and sometimes a guide to treatment. Every physician should be familiar with the normal range of these constituents as well as the significance of variations from normal.

As a general rule, the blood should be drawn before breakfast because the process of digestion and absorption of certain products by the blood influences the level of many determinations. It should also be recognized that in-vitro changes occur in the level of some constituents, especially in glucose; hence, there should be no delay in carrying out the tests.

Chemical determinations are carried out on whole blood, plasma, or serum. For whole blood or plasma, an anticoagulant, such as sodium oxalate, is used. To procure blood for serum, no anticoagulant is used. From 3 to 5 cc. of blood is sufficient for most tests.

Normal Values:
Blood-chemistry determinations are not exact; but if carried out carefully, they are of definite clinical value. Normal values are based on a series of determinations that give the average range in normal persons.

TABLE 1: NORMAL VALUES OF BLOOD CONSTITUENTS

	Material Required for Analysis	*Range per 100 Cc.*
Albumin	Plasma	3.8 to 5.2 gm.
Albumin-globulin ratio	Plasma	1.5 to 2.5:1
Alkali reserve	Plasma	53 to 75 volumes per cent (infants about 10 per cent lower)
Amylase	Serum	70 to 200 mg. of glucose from starch by 100 cc. serum
Bilirubin	Serum	0.1 to 0.25 mg.
Bromine	Serum	0.33 to 1.73 mg.
Calcium	Serum	9 to 11 mg.
Chlorides (as Cl)	Serum	352 to 383 mg.
Chlorides (as NaCl)	Plasma	570 to 620 mg. (100 to 106 mEq. per liter)
Cholesterol:		
Cholesterol ester	Plasma	190 to 200 mg.
Cholesterol, free	Plasma	40 to 50 mg.
Cholesterol, total	Plasma	130 to 250 mg.
Copper	Whole blood	0.14 to 0.15 mg.
Creatinine		1 to 2 mg.
Fat, neutral	Plasma	0 to 350 mg.
Fatty acids	Plasma	350 to 450 mg.
Fibrinogen	Plasma	0.2 to 0.4 gm.
Globulin	Plasma	1.5 to 3.4 gm.
Glucose	Whole blood	80 to 110 mg.
Glucose tolerance (standard test):		
One-half hour		120 to 130 mg.
One hour		140 to 170 mg.
Two hours		100 to 120 mg.
Three hours		80 to 100 mg.
Icterus index	Serum	4 to 6 units
Iodine	Whole blood	1.5 to 12.8 micrograms
Iron, inorganic	Serum	0.8 to 0.17 mg.
Lactic acid	Plasma	3 to 20 mg.
Lipase	Serum	1 to 1.5 cc. N/20 Sodium hydroxide
Lipoids, total	Plasma	400 to 600 mg.
Magnesium	Serum	1.85 to 2.09 mg.
Nitrogen:		
Amino-acid nitrogen	Whole blood	5 to 8 mg.
Ammonia nitrogen	Whole blood	0.1 to 0.2 mg.

TABLE 1: *(Continued)*

	Material Required for Analysis	*Range per 100 Cc.*
Nitrogen—Continued		
Nonprotein nitrogen	Whole blood	25 to 35 mg.
Undetermined nitrogen	Whole blood	5 to 18 mg.
Urea nitrogen	Whole blood	12 to 18 mg.
Nonprotein nitrogen	Whole blood	25 to 35 mg.
Phosphatase:		
Acid, phosphatase	Serum	0.5 to 2 Gutman units*
Alkaline, phosphatase	Serum	Adults: 1.5 to 4 Bodansky units† Children: 4 to 14 Bodansky units
Phospholipids	Plasma	60 to 350 mg.
Phosphorus (as inorganic phosphate)	Serum	Adults: 3.7 mg. Children: 5.6 mg.
Potassium	Serum	16 to 22 mg. (4.1 to 5.6 mEq. per liter)
Protein, total	Plasma	6.3 to 8 gm.
Albumin	Plasma	3.8 to 5.2 gm.
Albumin-globulin ratio	Plasma	1.5 to 2.5 : 1
Fibrinogen	Plasma	0.2 to 0.4 gm.
Globulin	Plasma	1.5 to 3.4 gm.
Sodium	Serum	315 to 340 mg. (136 to 145 mEq. per liter)
Uric acid	Whole blood	2 to 4 mg.
Urea	Whole blood	20 to 30 mg.
Urea nitrogen	Whole blood	12 to 18 mg.
van den Bergh, quantitative	Serum	0.1 to 0.25 mg.
Vitamin C	Plasma	0.7 to 1.4 mg.

* A Gutman unit is the amount of phosphatase required to liberate 1 mg. per cent of phenol after one hour of incubation at 37° C. using disodium phenyl phosphate at *p*H 4.9 in the substrate.

† A Bodansky unit is the amount of phosphatase required to liberate 1 mg. of inorganic phosphate per 100 cc. of serum after one hour of incubation at 37° C. and a *p*H of 8.6 with the substrate containing sodium beta glycerophosphate.

GLUCOSE

Physiology of Glucose Metabolism:

When complex carbohydrates, such as sugars and starches, are ingested, they are changed into monosaccharides by the various enzymes. They are acted upon by the ptyalin of the saliva, the amylase from the pancreas, and specific intestinal enzymes, such as maltase, invertase, and lactase. The most important monosaccharide formed is glucose, or dextrose. Other monosaccharides formed in smaller amounts are levulose and galactose. Glucose is also derived from certain amino acids and from lactic acid released by the muscles. This is changed into glycogen in the liver and later into glucose.

Glucose is brought to the liver by the portal circulation, chiefly from the small intestine. The excess is stored in the liver as glycogen (glycogenesis). Some glucose passes through the liver as such and is changed into glycogen and stored in the muscles.

Glycogen stored in the liver is changed, when needed, into glucose (glycogenolysis). Thus, the liver serves as a reservoir of glucose to keep the blood glucose at a normal level. When normal blood contains an excessive amount of glucose, as after a meal, it is withdrawn from the blood and deposited in the liver as glycogen and is also converted into fat in the tissues.

Insulin performs certain important functions in connection with glucose metabolism. Most important is its function in connection with the storage of glycogen in the liver and the control of glycogenolysis. It also increases the oxidation of glucose, enhances the deposition of glycogen in muscle, and decreases the catabolism of protein and fat. This brings about a decrease in glucose formation from amino acids and also a decrease in ketone-body formation from hepatic fatty acids.

The normal fasting blood-glucose level is from 80 to 110 mg. per 100 cc. in adults. The normal range for the true glucose values after excluding the nonglucose-reducing products is from 70 to 100 mg. per 100 cc.

Hyperglycemia:

An increase in blood glucose is noted under certain physiological conditions, as during digestion and after muscular exercise. It is an important guide in the diagnosis and treatment of diabetes mellitus. Some increase in blood sugar is also found in vomiting of pregnancy, anesthesia, asphyxia, pneumonia, nephritis, dehydration, and increased intracranial pressure. There is a slight hyperglycemia in certain endocrine disturbances, as in early hyperpituitarism, hyperthyroidism, and hyperadrenalism.

There is, of course, an increase in blood glucose during and after intravenous infusion of glucose solution. For a proper evaluation, the blood-glucose level should be determined when the patient is fasting (preferably before breakfast) and when it is not influenced by intravenous solutions.

Hypoglycemia:

A decrease in blood glucose below the lower limit of normal is called "hypoglycemia." It is decreased in certain physiological conditions, such as pregnancy, lactation, starvation, and after severe, prolonged, muscular exercise. A decrease is noted in hyperinsulinism, which may be due either to an overdosage of insulin or to excessive insulin production by a tumor of the islets of the pancreas. There is some hypoglycemia in arthritis, marked anemia, chronic nephritis with renal failure, late stages of pregnancy, hypoadrenalism, and in some cases of glycogenosis and obesity.

Increased Tolerance:

Persons showing little or no increase in blood-glucose values for two hours following ingestion of a standard amount of glucose have an increased tolerance. Increased tolerance is found in hyperinsulinism resulting from overdosage of insulin or from excessive insulin formation by an adenoma or carcinoma of the islands of Langerhans. Increased sensitivity to insulin in normal amounts also causes increased tolerance. Increased tolerance to glucose is also noted when there is a decrease in insulin antagonists, as in hypothyroidism (cretinism, myxedema), hypoadrenalism (Addison's disease), and hypopituitarism (Simmonds' disease, congenital defects, and certain tumors).

Glucose-Tolerance Tests:

Standard Test: The standard test involves one dose of glucose administered by mouth. The test is carried out in the following manner:

1. A sample of blood and a sample of urine are taken in the morning from the patient, who has been fasting since supper the previous evening.
2. A solution of 100 gm. of glucose in 300 cc. of water and flavored with lemon juice is given by mouth.
3. Blood and urine specimens are taken at the end of one-half hour, one hour, two hours, and three hours.

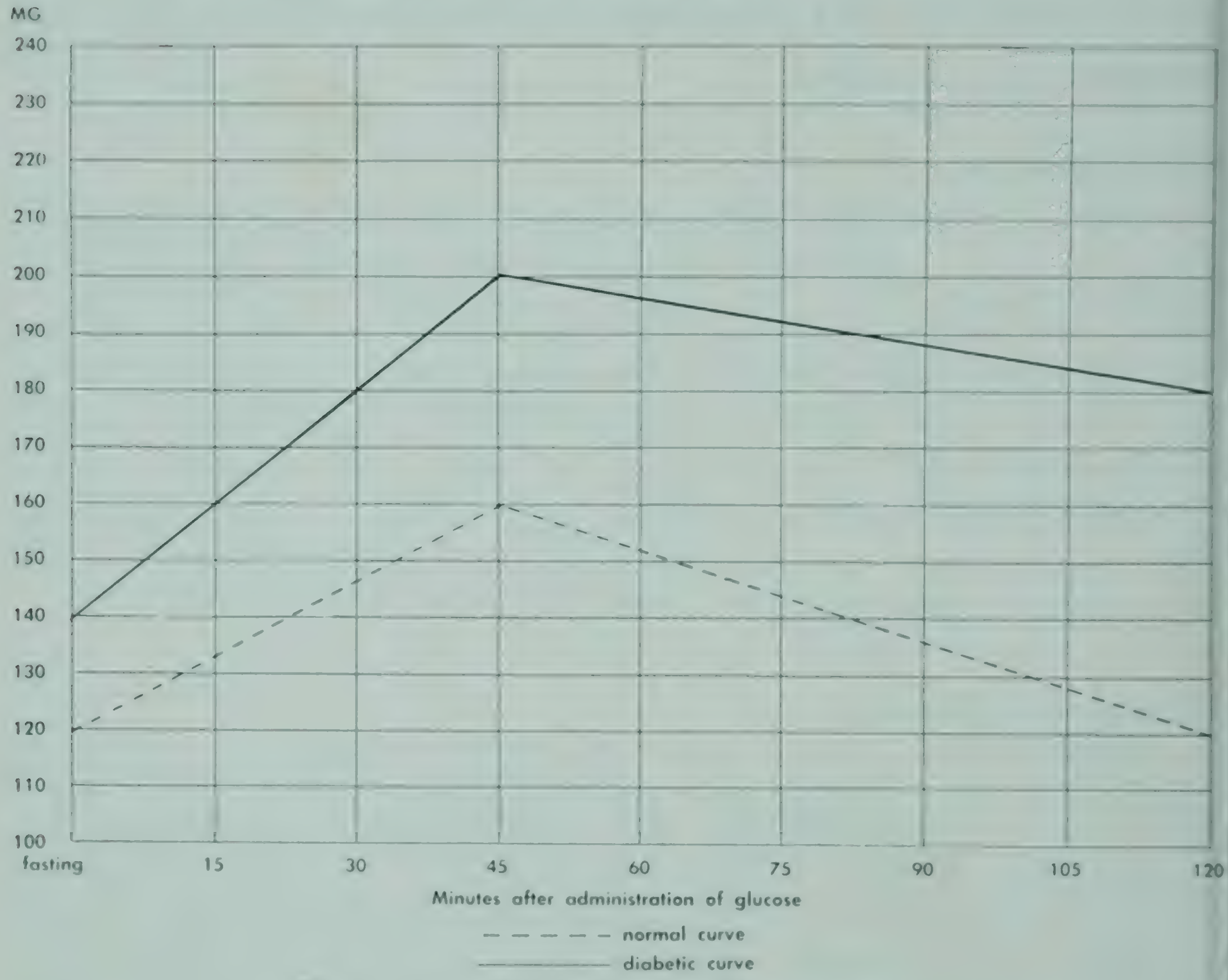


Fig. 1: Standard glucose-tolerance test.

A normal person has a fasting blood-sugar level of from 80 to 120 mg. per 100 cc., an increase at the end of one hour, and return to normal at the end of two hours. All urine specimens except the one-hour specimen should be negative for glucose. The one-hour specimen may or may not show a positive test. All but the first urine specimen are positive for glucose in renal glycosuria, but the blood-sugar levels follow a normal curve.

A diabetic person has a fasting blood-sugar level of 120 mg. per 100 cc. or higher. At the end of an hour, the level is higher than

180 mg. per 100 cc. and does not return to normal in the two- and three-hour specimens (Fig. 1). The urine is usually positive for glucose in the second, third, and fourth specimens.

The Exton-Rose, Glucose-Tolerance Test: The Exton-Rose test[1] has certain advantages over the standard test. It takes only one hour and requires only three specimens of blood and urine. Some physicians believe it is more specific than the standard test. The procedure is as follows:

1. A sample of blood and a sample of urine are taken in the morning from the patient, who has been fasting since supper the previous evening.
2. A solution of 100 gm. of glucose in 650 cc. of water flavored with lemon juice is prepared. One half of this solution is given by mouth.
3. Thirty minutes later, another sample of blood and a sample of urine are taken, and the second half of the solution is given.
4. Thirty minutes later, samples of blood and urine are taken.

In this test, a normal curve is characterized by a normal, fasting, blood-glucose level—a response of not over 160 mg. per 100 cc. at the end of one-half hour and a drop below the one-half-hour level at the end of one hour. This test in a diabetic person is characterized by a normal or increased, fasting, blood-glucose level, an increase of more than 25 or 30 mg. in the second specimen (end of one-half hour), and a blood-glucose level above 160 mg. in the third specimen (end of one hour) (Fig. 2).

Glucose-Tolerance Test for Infants: A method for testing glucose tolerance in children under two years is given by Livingston and Bridge[2] as follows:

1. The child is kept on a normal diet.
2. A twelve-hour fast is required for infants receiving four or five feedings a day, and a nine-hour fast for those receiving six or more feedings and for premature infants.
3. A solution of 3 gm. of glucose per kilogram of body weight in from 30 to 60 cc. of orange juice is given after taking a fasting blood specimen.
4. Blood specimens are taken one-half hour, one, two, and three hours after administering the glucose. Capillary blood is used, and the examination is carried out by a microchemical method.

5. The intravenous method may be used, in which 1 gm. of glucose per kilogram of body weight made up in a 50 per cent solution is given.

The normal, fasting, blood-sugar level for an infant is approximately 90 mg. per 100 cc., but on a fat-high diet it will be as low as 70 mg. The level in a normal infant shows from 160 to 180 mg. at the height of the curve and returns to normal at the end of three hours. The level in a diabetic infant will show higher values at the peak of the curve and will not return to normal in three hours.

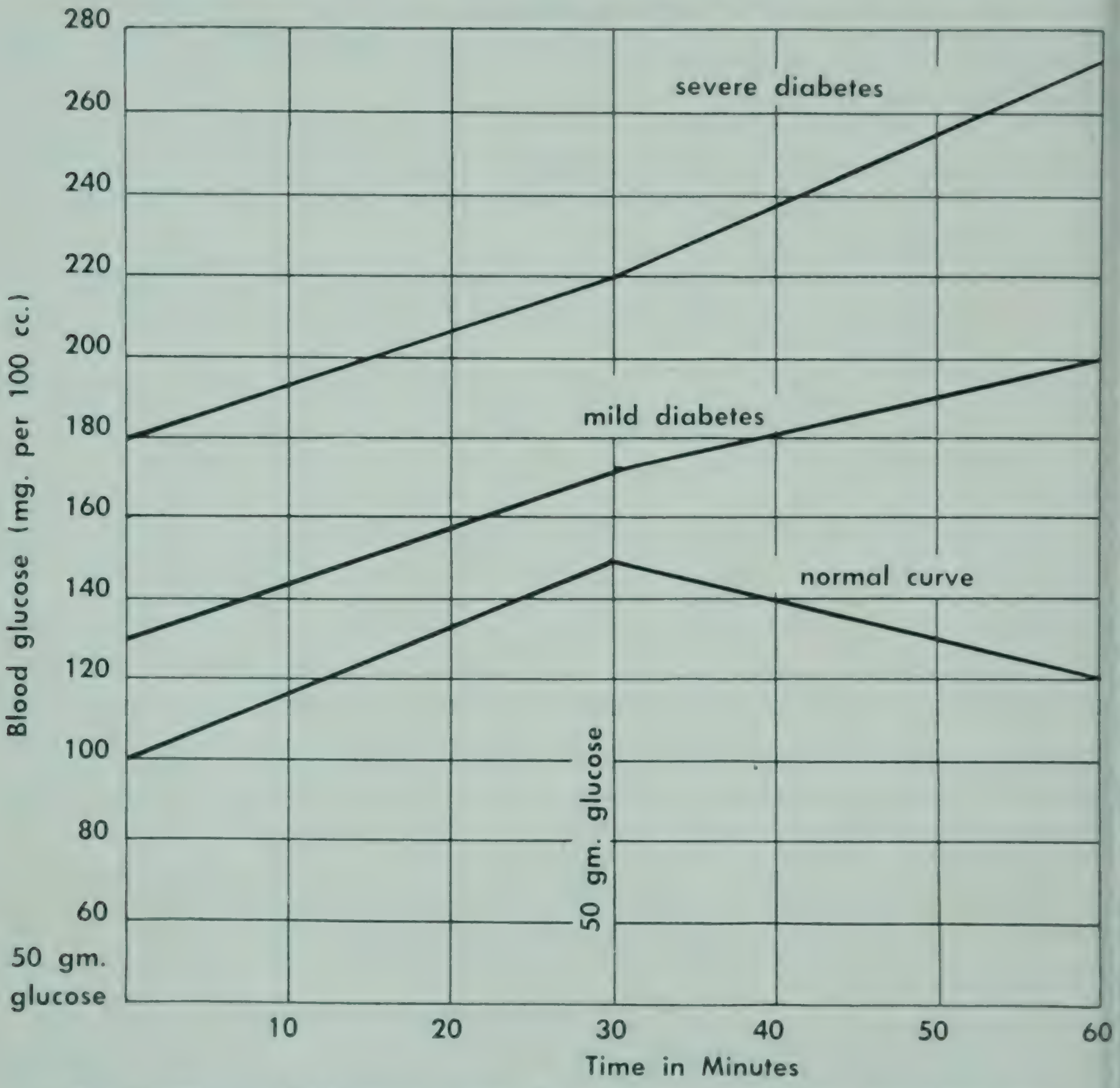


Fig. 2: Glucose-tolerance curve. Exton-Rose, one-hour, two-dose test.

NITROGENOUS COMPOUNDS

The nitrogenous compounds of the blood consist of nonprotein nitrogenous compounds and plasma proteins. The nonprotein nitrogenous compounds consist of urea, uric acid, creatinine, amino acids, ammonia, and the so-called "rest nitrogen." *Urea* is formed from ingested proteins, which are broken up by proteolytic enzymes into proteoses, peptins, peptones, and amino acids. The *amino acids* are carried to the liver, where they are synthesized into urea. *Urea* is also one of the chief products of tissue-protein metabolism. *Uric acid* is the product of both protein digestion and tissue metabolism, while *creatinine* is produced only by tissue-protein metabolism. As indicated, amino acids are the chief end-products of protein metabolism and are carried to the liver where they are deaminized and synthesized into urea. *Ammonia* is an undeaminized product of amino acids. *Rest nitrogen* is found for the most part in the blood corpuscles, but the exact method of its formation and its significance are not definitely known.

Nonprotein Nitrogen

The amount of normal nonprotein nitrogen is from 25 to 35 mg. per 100 cc. It is made up of the nitrogen of urea, uric acid, creatinine, amino acids, ammonia, and rest nitrogen.

Urea Nitrogen:

Urea is formed almost entirely by the liver from both protein digestion and protein metabolism in the tissues. Urea values normally range from 20 to 30 mg. per 100 cc. At present, however, the preferred technic is to determine the urea nitrogen that has normal values of from 12 to 18 mg. per 100 cc. It is important that urea nitrogen be determined only on fasting persons since there is an increased blood level following ingestion of protein. Increased urea-nitrogen values, in general, parallel those of nonprotein nitrogen. In certain liver diseases, however, there is likely to be a more evident decrease of urea nitrogen than of nonprotein nitrogen.

Creatinine:

The normal creatinine values are from 1 to 2 mg. per 100 cc. They are increased in those conditions in which nonprotein nitrogen and urea are increased. But there is frequently a lag in the increase of creatinine; consequently, when it is increased, particularly in chronic nephritis, it makes the prognosis more serious.

Conditions Causing Increase in Nonprotein Nitrogen, Urea, and Creatinine:

Renal Disease: Acute, subacute, and chronic nephritis; toxic nephroses; uremia; postoperative suppression of kidney function; tuberculosis of kidneys; peripheral cortical necrosis; polycystic kidneys; pyonephrosis; pyelonephritis (marked); hydronephrosis; amyloidosis; congenital hypoplasia of kidneys; hemoglobinuria; and bilateral obstructing kidney stones.

Prerenal Azotemia: Severe heart failure, vomiting, diarrhea, intestinal obstruction, high fevers, gastric tetany, dehydration, severe burns, crises of Addison's disease, diabetic acidosis, manic-depressive psychosis in extreme excitation, hyperthyroidism, pancreatic necrosis, multiple myeloma, profuse hemorrhage, and shock.

Postrenal Azotemia: Prostatic hypertrophy with obstruction, bilateral ureteral obstruction, urethral stricture with obstruction.

Conditions Causing Decrease in Urea:

The following conditions cause a low normal level of urea or a decrease in urea value, but nonprotein nitrogen and creatinine do not decrease significantly:

Liver Disease: Toxic hepatitis; eclampsia; phosphorus, arsenic, chloroform, and carbon-tetrachloride poisoning; acute yellow atrophy; biliary-tract operations; and rarely in cirrhosis.

Miscellaneous Conditions: Protein low-diet (sometimes late in pregnancy), lipoid nephrosis, celiac disease, and acromegaly.

Uric Acid:

The normal range of uric acid is from 2 to 4 mg. per 100 cc. Uric acid is probably stored in the kidneys and is released from these organs to be destroyed, particularly by the liver. A large part of the uric acid is also excreted by the kidneys. Consequently, there is an increase in the blood level in liver disease, particularly eclampsia, and in acute and chronic nephritis.

Uric acid is also increased in gout. The exact nature of the abnormal metabolism of proteins is not known; but in susceptible persons, it is brought about by a diet rich in purine foods. It is not known whether the liver is unable to destroy the uric acid or the kidneys are unable to store and excrete uric acid. In any event, during attacks of the disease, uric acid is stored in the cartilages, particularly in the great toes, which become very painful.

Conditions Causing Increase in Uric Acid:

Liver Disease: Eclampsia and acute hepatitis.

Metabolic Disease: Gout and rheumatoid arthritis.

Renal Disease: Acute and chronic nephritis before a rise in nonprotein nitrogen, urea, and creatinine; renal tuberculosis; pyelonephritis; pyonephrosis; hydronephrosis; and reflex anuria.

Miscellaneous Conditions: Multiple myeloma, myelogenous and lymphocytic leukemia, remission of pernicious anemia, obstruction of ureters, prostatic obstruction, lead poisoning, intestinal obstruction, hypertension, severe congestive heart failure, lobar pneumonia, and some cases of chronic dermatitis.

Conditions Causing Decrease in Uric Acid:

A decrease in uric acid is not of particular diagnostic aid because it is low normal or only slightly decreased in celiac disease and in relapses of pernicious anemia.

Amino Acids:

The normal blood level of amino acids is from 5 to 8 mg. per 100 cc. Amino acids are deaminized in the liver to produce urea. Consequently, when the function of the liver is not normal, the amino acids are increased in the blood.

Amino acids are increased in phosphorus, arsenic, chloroform, and carbon-tetrachloride poisoning; catarrhal jaundice; eclampsia; myelogenous leukemia; some cases of chronic nephritis; and in obstruction of the urinary tract.

Ammonia and rest nitrogen are not of any great clinical significance. Determinations of amino acids, ammonia, and rest nitrogen are not usually included in routine clinical investigation.

PLASMA PROTEINS

The blood-plasma proteins consist of albumin, globulin (euglobulin and pseudoglobulin), and fibrinogen. The normal albumin values are from 3.8 to 5.2 gm.; globulin, from 1.5 to 3.4 gm.; and fibrinogen, from 0.2 to 0.4 gm. per 100 cc. The sum of these, or total-protein values, ranges from 6.3 to 8 gm. per 100 cc. The albumin-globulin ratio is from 1.5 to 2.5 : 1.

The site of formation of albumin and globulin is not known, but it seems reasonable to assume that they are formed in those organs from which they may diffuse readily into the bloodstream, for example the spleen, bone marrow, and liver. Fibrinogen appears to be formed only in the liver.

Albumin and Globulin:

Albumin and globulin maintain a normal colloid osmotic pressure in the capillaries, and thus keep up a proper balance between the plasma and tissue fluids. Since the plasma has a higher protein content than tissue fluids it would be expected that tissue fluids would pass into the plasma by osmosis until both fluids attain the same density. The hydrostatic pressure in the arterial capillaries, however, prevents the passage of fluid from the tissues. On the contrary, this hydrostatic pressure normally causes a flow of fluid from the arterial capillaries to the tissues.

To maintain the proper balance of fluid, there is also a flow of fluid from the tissues to the bloodstream through the venules. This takes place because of the low hydrostatic pressure within the venules and because of the greater concentration of proteins.

In a state of decreased blood proteins (hypoproteinemia), there is a reduction in the osmotic pressure in both venous and arterial capillaries, which contributes to the development of tissue edema. In hypoproteinemia, the albumin usually shows a greater drop than globulin does; in fact, there may be a compensatory increase in globulin in certain diseases.

Hyperproteinemia: An increase in total protein value is found in the following:

1. Conditions causing a loss of fluid: diarrhea in infants, vomiting from high intestinal obstruction, dehydration from inadequate

fluid intake, and loss of fluid from the surfaces of extensive burns.
2. Addison's disease.

HYPERPROTEINEMIA DUE TO INCREASED GLOBULIN: An increase in total protein value due chiefly to increase in globulin is found in the following:

1. Infections: active pulmonary and extrapulmonary tuberculosis, pneumonia, bacterial endocarditis, rheumatoid arthritis, lung abscess, osteomyelitis, lymphogranuloma venereum, syphilis, leprosy, lupus erythematosus, dermatomyositis, and Boeck's sarcoid.
2. Parasitic diseases: malaria, visceral leishmaniasis (kala-azar), filariasis, trypanosomiasis, and schistosomiasis.
3. Neoplastic processes: multiple myeloma, myelogenous and lymphocytic leukemia, and sarcoid of Boeck.

Hypoproteinemia: A decrease in total-protein value involves chiefly a reduction of albumin. A total-protein value of less than 5.5 gm. per 100 cc. favors the formation of edema. Hypoproteinemia occurs in the following:

1. Diseases of the kidneys involving prolonged, marked albuminuria; lipoid nephrosis.
2. Diseases of the liver: cirrhosis, toxic hepatitis, acute yellow atrophy, and eclampsia.
3. Diseases of the intestinal tract causing prolonged diarrhea or vomiting.
4. Diseases causing malnutrition, such as carcinoma, tuberculosis, and prolonged anemia.

Reversal of Albumin-Globulin Ratio: The normal albumin-globulin ratio is from 1.5 to 2.5:1. This ratio is reversed in those diseases that cause a loss of albumin and a compensatory increase in globulin. The important diseases that bring about such a reversal are lipoid nephrosis and cirrhosis of the liver with ascites.

Fibrinogen:

The normal values for fibrinogen are from 0.2 to 0.4 gm. per 100 cc. It is transformed into fibrin by thrombin and thus brings about coagulation of the blood.

Fibrinogen is increased in the following:

1. Lipoid nephrosis.
2. Infections: septicemia, pneumonia, mild hepatitis and cholecystitis, pulmonary tuberculosis, and prolonged local infectious processes.
3. After x-ray treatment and during pregnancy and menstruation.

Fibrinogen is decreased in the following:

1. Liver diseases (cirrhosis and acute yellow atrophy); arsphenamine, chloroform, phosphorus, and carbon-tetrachloride poisoning.
2. Severe malnutrition, typhoid fever, marked anemia, and for a short time after extensive hemorrhage.

PHYSIOLOGY OF ACID-BASE EQUILIBRIUM

The normal *p*H of the blood is 7.4, which is slightly alkaline (*p*H of 7 is neutral). The normal, plasma, bicarbonate, or alkali, reserve is from 53 to 75 volumes per cent. The neutralization of acid and removal of excess acid—that is the maintenance of the proper reaction, or *p*H—is carried out, according to Best and Taylor,[3] by the following:

1. The buffer systems of the blood (see p. 543).
2. Excretion of carbon dioxide by the lungs (see p. 544).
3. Excretion of fixed acid by the kidney (see p. 544).
4. Removal of acid, particularly phosphoric, by the intestinal mucosa.

Alkali Reserve:

The alkali reserve is the amount of base found as bicarbonate in the blood, which is available for the neutralization of fixed acids, such as lactic acid and hydrochloric acid. It should be made clear that alkali reserve refers only to base bound as bicarbonate and not to the total base of the blood. The normal plasma bicarbonate is a measure of base left over after all acids stronger than carbonic acid have been neutralized; thus, it indicates the reserve of alkali readily available for the neutralization of such acids.

The plasma bicarbonate may be greatly depleted; yet if the free carbonic acid is reduced to a corresponding extent and the normal ratio of 1:20 (carbonic acid: bicarbonate) is thereby maintained, the *p*H will show no appreciable change. Thus, the *p*H of the plasma depends upon the ratio between carbonic acid and sodium bicarbonate.

The *p*H of the blood never becomes acid in disease conditions, except occasionally in diabetic coma when it may reach 6.8. The limits of *p*H of the blood that are compatible with life are from 6.8 to 7.8. The respiratory center is particularly sensitive to slight variation in carbon-dioxide tension and to changes in the *p*H of the blood. The elimination of carbon dioxide through the lungs is the most important of the mechanisms for keeping the *p*H within normal range.

Acidosis: Acidosis (see also p. 548) is a condition of decreased alkali reserve resulting from formation of acids faster than they can be neutralized or from loss of base. Acidosis is not necessarily associated with acidity of the blood, that is a *p*H below 7, except in extreme cases of diabetic acidosis. Because of this lack of change in *p*H, the status of acidosis or alkalosis is indicated better by the determination of the alkali reserve. The normal range is from 53 to 75 volumes per cent.

The alkali reserve is decreased in the following:

1. Severe diabetes mellitus, when the oxidation of fatty acid is not complete and there is an excess of ketone bodies (acetone, diacetic acid, β-hydroxybutyric acid) (coma usually occurs when it is reduced to from 28 to 10 volumes per cent).
2. Kidney disease in which there is failure to excrete fixed acid: acute nephritis, nephrosis, pyelonephritis, pyonephrosis, tuberculosis of kidneys, and polycystic kidneys.
3. Prolonged obstruction of the lower urinary tract, as in hypertrophy of the prostate.
4. Loss of fluids: excessive vomiting and diarrhea.
5. Infections: bronchopneumonia and terminal pulmonary tuberculosis.
6. Excessive carbon dioxide: inhalation of carbon dioxide, asphyxia, anesthesia, emphysema, severe asthma, and congestive heart failure.
7. Excessive morphine dosage.
8. Toxemia of pregnancy.

Alkalosis: Alkalosis (see also p. 549) is a condition of increased alkali reserve. The *p*H of the blood is not necessarily affected by an increase in the alkali reserve. Under certain conditions, it may be increased to 100 volumes per cent or more.

The alkali reserve is increased in the following:

1. Conditions resulting in a loss of hydrochloric acid, such as vomiting from high intestinal or pyloric obstruction, pylorospasm and toxicoses of the intestinal tract of infants and children, general peritonitis with vomiting, and uremia with vomiting.
2. Ingestion of large quantities of sodium bicarbonate.
3. Exposure to high temperature and to high altitudes.
4. Hot baths.
5. Prolonged treatment with radium, x-rays or ultraviolet light.

Lipoids:

The lipoids of the blood include neutral fat, fatty acids, phospholipids (or phosphatides), and sterols. Neutral fat and fatty acids are derived from ingested fats, which are changed to fatty acids and glycerol by lipase and bile salts. Some neutral fat escapes into the lacteals and then to the bloodstream, where it makes the plasma milky following meals. Fatty acids are also absorbed into the lacteals, but they are changed back to a form of neutral fat that is stored in the fat depots.

Phosphatides are ingested in foods and apparently may be synthesized from fat. Phosphatides are broken up by lipase and after absorption are resynthesized again into phosphatides. They are excreted mostly in the bile and intestinal secretions.

The important sterol is cholesterol. Cholesterol is derived from the tissues (endogenous) and from ingested food (exogenous). The endogenous cholesterol is thought to be controlled by the reticulo-endothelial system. The liver is an important organ in cholesterol metabolism because it stores the substance and excretes cholesterol esters into the plasma. Cholesterol occurs in the plasma as free cholesterol and as cholesterol esters.

Hyperlipemia: The normal range for neutral fat in plasma is from 0 to 370 mg. per 100 cc., and for fatty acids from 350 to 450 mg. per 100 cc.

An increase in fat and fatty acids is called "hyperlipemia." It occurs in the following:

1. Dietary states: after meals, after fasting, after prolonged exclusive meat diet and, starvation.

2. Metabolic disorders: hypothyroidism, hypertension, diabetic acidosis, and glycogenosis.
3. Blood disorders: pernicious anemia, hypochromic anemia, hemolytic anemia, and leukemia.
4. Renal disease: glomerulonephritis and nephrosis.
5. Drugs: ether anesthesia and alcohol.

Hypolipemia: A decrease in fat and fatty acids is called "hypolipemia." It occurs in hyperthyroidism.

Hyperphospholipidemia: The normal range of phospholipids is from 60 to 350 mg. per 100 cc. of plasma. Hyperphospholipidemia is an increase in phospholipids. It occurs in the following:

1. Malnutrition associated with advanced diabetes mellitus.
2. Vitamin-B deficiency.
3. Secondary anemia from chronic hemorrhage.
4. Glomerulonephritis and nephrosis.
5. Lipoid histiocytosis (Niemann's disease).

Hypophospholipidemia: A decrease in plasma phospholipids is called "hypophospholipidemia." It occurs in the following:

1. Anemia, idiopathic hypochromic anemia, hemolytic anemia with jaundice, and pernicious anemia in relapse.
2. Acute infections with fever.
3. Hyperthyroidism on occasion.

Hypercholesterolemia: The normal range of total plasma cholesterol is from 130 to 250 mg.; free cholesterol, from 40 to 50 mg.; and cholesterol esters, from 190 to 200 mg. per 100 cc. An increase in cholesterol is called "hypercholesterolemia." It occurs in the following:

1. Metabolic disorders: hypothyroidism, severe diabetes mellitus, atherosclerosis, celiac disease, xanthomatosis, cataract, familial splenic anemia (Gaucher's disease), lipoid histiocytosis, glycogenosis, and hypertrophic osteoarthritis.
2. Hepatobiliary disease: hepatocellular disease with jaundice, obstruction of common bile duct, and biliary fistula.
3. Physiological states: normal pregnancy and before menstruation.
4. Renal disease: chronic glomerulonephritis, lipoid nephrosis, and amyloid disease.
5. Sudden severe hemorrhage.

6. Psoriasis.
7. Ether anesthesia.

Hypocholesterolemia: A decrease in plasma cholesterol is called "hypocholesterolemia." It occurs in the following:

1. Metabolic disorders: hyperthyroidism, cachexia, arteriosclerosis, coronary-artery thrombosis, celiac disease, and congestive heart failure.
2. Liver diseases: portal cirrhosis (terminal) and hepatocellular damage (this causes a decrease particularly in cholesterol esters).
3. Blood diseases: pernicious anemia, hemolytic jaundice, and marked hypochromic anemia.
4. Infectious diseases: pulmonary tuberculosis and acute pancreatitis.
5. Urinary tract disease: obstruction of the urinary tract and nephrotic type of terminal chronic glomerulonephritis.
6. High intestinal obstruction.
7. Certain cases of polyneuritis, particularly with malnutrition.

BILIRUBIN

Origin:

Bilirubin is formed from hemoglobin by the reticuloendothelial system through the process of hydrolysis. It is formed chiefly by the reticuloendothelial cells of the spleen, bone marrow, and Kupffer's cells of the liver. The polygonal cells of the liver withdraw the bilirubin from the blood and excrete it in the bile. It should be recognized that there is a difference between the bilirubin before (prehepatic) and after (posthepatic) it has passed through the liver cells. Bilirubin that has passed through the polygonal cells of the liver is more dialyzable, more oxidizable, more readily absorbed by precipitated protein, and is not soluble in chloroform.

The qualitative, direct and indirect, van den Bergh reactions are used to differentiate between prehepatic and posthepatic bilirubin when present in excessive amounts. These reactions thus help to differentiate between hemolytic and obstructive jaundice. In hemolytic jaundice, there is excessive destruction of red blood cells with an excessive amount of bilirubin. The liver cells are unable to withdraw the bilirubin from the blood as fast as it is formed. There is, consequently, an increase in prehepatic bilirubin, which gives a positive, indirect, van den Bergh reaction. In obstructive jaundice, either extrahepatic or intrahepatic, the bilirubin is reabsorbed into the bloodstream after it has passed through the liver cells. This posthepatic bilirubin gives a direct van den Bergh test.

van den Bergh Reaction:

The van den Bergh test is a modification of Ehrlich's diazo reaction and is employed to detect the type of bilirubin in the blood serum.

Solution A

Sulfanilic acid	0.1	gm.
Concentrated hydrochloric acid	1.5	cc.
Water	q. s. ad 100.0	cc.

Solution B

Sodium nitrite	0.5	gm.
Water	100.0	cc.

In performing the test, 10 cc. of Solution A and 0.3 cc. of Solution B are mixed just before using. The *direct reaction* takes place when 1 cc. of the fresh reagent is added to 1 cc. of serum. The following types of reaction may take place: (1) *immediate or prompt* (a violet color due to the formation of diazobilirubin develops in from twenty to thirty seconds); (2) *delayed reaction* (no change takes place until after a minute or more, then a reddish color develops, which gradually becomes violet); and (3) *biphasic* (a red color develops promptly and takes a variable length of time to change to violet). Serum bilirubin in obstructive jaundice gives an immediate, direct reaction.

The *indirect reaction* requires the use of alcohol; 1 cc. of serum is mixed with 2 cc. of 95 per cent alcohol. The mixture is shaken and centrifuged, then 0.25 cc. of the fresh mixture and 0.5 cc. of 95 per cent alcohol are added to 1 cc. of the supernatant fluid. The serum of hemolytic jaundice and normal serum give a positive, qualitative, indirect, van den Bergh test.

Quantitative estimations of bilirubin may be made with the indirect reaction. After the color reaction has taken place, it is compared in a colorimeter with a standard solution of 2.161 gm. of anhydrous cobaltous sulfate in 100 cc. of distilled water. The color of this standard corresponds to that developed by 1 unit of bilirubin. A unit is defined by van den Bergh as one part of bilirubin in 200,000 parts of serum. Normal values are from 0.4 to 2 units, or from 0.2 to 1 mg. per 100 cc. of blood.

Icterus Index:

The icterus index is also used to determine an increase of bilirubin in the serum. This test compares the color of serum with a standard 1:10,000 solution of potassium bichromate, and the results are expressed in units. The normal range is from 4 to 6 units, but it may reach 15 units before clinical jaundice is evident. With values above 16 units, jaundice can be detected clinically. An icterus index of 10 units corresponds approximately to 1 mg. of bilirubin, according to the indirect van den Bergh reaction. This, of course, assumes that the serum is not colored by other pigments, such as carotene.

For an accurate determination of the icterus index, the serum should be free of hemolyzed red cells and chyle.

Hyperbilirubinemia:

An increase of bilirubin in the serum is called "hyperbilirubinemia." This may be due to prehepatic bilirubin, bilirubin excreted by the liver, or a mixture of the two. It occurs as follows:

1. Prehepatic bilirubinemia:
    - *a.* Hemolytic jaundice, congenital and acquired
    - *b.* Paroxysmal hemoglobinuria
    - *c.* Pernicious anemia
    - *d.* Sickle-cell anemia
    - *e.* Polycythemia
    - *f.* Icterus neonatorum
    - *g.* Internal hemorrhage
    - *h.* Hemolysis following transfusion
    - *i.* Acute hemolytic anemia
    - *j.* Malaria
    - *k.* Oroya fever
    - *l.* Septicemia, streptococcus or staphylococcus
    - *m.* Fasting
    - *n.* Increased blood formation and destruction in high altitudes
2. Posthepatic bilirubinemia (bilirubin excreted by the liver):
    - *a.* Extrahepatic obstruction of biliary system (hepatic and common ducts) by calculi, tumors, adhesions, kinks, or external pressure

   - *b.* Intrahepatic obstruction of bile ducts by tumors, cholangitis, portal and biliary cirrhosis, and parasites
   - *c.* Hepatitis resulting from yellow fever, catarrhal jaundice, leptospiral jaundice, and syphilis
   - *d.* Toxic hepatitis resulting from chloroform, arsphenamine, cinchopen, phosphorus, eclampsia, carbon tetrachloride, and x-rays
   - *e.* Acute yellow atrophy
   - *f.* Celiac disease

Hypobilirubinemia:

A decrease in serum bilirubin is called "hypobilirubinemia." It occurs in the following:

1. Aplastic anemia.
2. Certain secondary anemias, particularly those resulting from the toxic agents of carcinoma and chronic nephritis.

CHLORIDES

Chlorides occur chiefly in the plasma in the form of sodium chloride. The erythrocytes contain half as much chloride as the plasma. The chlorides help to maintain acid-base equilibrium, proper osmotic equilibrium between the blood and tissues, and the fluid balance.

Chloride derived from the sodium chloride of the plasma diffuses into the red blood cells, and the base (sodium) thereby released combines with carbonic acid to form carbonate. This is referred to as the "shift of chlorides." Carbonic acid leaves the red blood cells and combines with the sodium freed from chloride to form plasma bicarbonate. Since these processes can take place in vitro when the blood is exposed to air, it is important to take the blood under oil and perform the test as soon as possible.

Sodium chloride is taken into the body with foods and is excreted primarily in the urine, and to a certain extent in the sweat and feces.

The normal plasma chloride, as sodium chloride, is from 570 to 620 mg. per 100 cc., or from 100 to 106 mEq. per liter. The normal serum chloride, as chloride ion, is from 352 to 383 mg. per 100 cc., or from 62 to 67 mEq. per liter.

Hyperchloremia:

An increase in plasma chloride is called "hyperchloremia." It occurs as follows:

1. As a result of decreased excretion:
    - *a.* In obstruction of urinary tract, ureters, urethral orifice, and urethra
    - *b.* In acute glomerulonephritis without excessive loss of fluid
    - *c.* In chronic glomerulonephritis with low intake of total protein and high intake of salt
    - *d.* Sometimes in nephrosis with low intake of total protein and high intake of salt
    - *e.* Sometimes in essential hypertension with chronic nephritis
    - *f.* Sometimes in the patient with a decompensated heart and edema
    - *g.* In pituitary cachexia
2. As a result of increased intake:
    - *a.* From excessive ingestion of salt, as salt tablets
    - *b.* After intravenous sodium chloride administration, particularly if urinary output is low
3. In hyperventilation states, such as hysteria

Hypochloremia:

A decrease in plasma chloride is called "hypochloremia." It occurs as follows:

1. As a result of abnormal loss of chloride:
    - *a.* In severe diarrhea in adults and infants
    - *b.* In severe vomiting from high intestinal obstruction, anemia, eclampsia, and pylorospasm
    - *c.* Fistula resulting in loss of gastric juice, pancreatic secretions, and bile
    - *d.* From withdrawal of ascitic fluid in hepatic cirrhosis with low salt intake
    - *e.* In excessive sweating from drinking of salt-free water
    - *f.* In nephrosis, particularly the type due to mercuric-chloride poisoning, resulting in failure of tubules to reabsorb sodium chloride
    - *g.* In advanced chronic nephritis, particularly with uremia
    - *h.* In severe diabetes mellitus with excessive urinary output and acidosis

   - *i.* In Addison's disease (increased excretion of chloride because of absence of adrenal cortical hormone)
2. As a result of decreased chloride intake:
   - *a.* In starvation
3. In emphysema (in those cases of increased carbon-dioxide tension in alveoli, there is a shift of chloride to red blood cells).
4. In certain infectious diseases, such as meningitis, rheumatic fever, pulmonary tuberculosis, and lobar pneumonia (the reason for decreased amount of chloride in these diseases is not known)
5. In some patients with hyperparathyroidism.

SODIUM

Sodium is normally absorbed from the intestinal tract into the bloodstream and is excreted in the urine. Most of the sodium of the body is in the serum in the form of sodium chloride. No sodium is present in the erythrocytes. It performs important functions in maintaining proper osmotic pressure and acid-base equilibrium. The normal sodium values in the serum are from 315 to 340 mg. per 100 cc., or from 136 to 145 mEq. per liter.

Sodium is increased in basophilic adenoma of the anterior pituitary gland (Cushing's syndrome). The increase of sodium in this disease may be due to increased function of adrenal cortex because of an excessive amount of circulating adrenotropic hormone.

Sodium is decreased in the following:

1. Addison's disease due to hypofunction and hyposecretion of the cortical hormone, causing excessive excretion of sodium together with chloride and water in the urine
2. Vomiting from high intestinal obstruction or pyloric stenosis, resulting in loss of chloride and some sodium
3. Fistula, resulting in loss of intestinal contents
4. Prolonged diarrhea
5. Excessive perspiration, as in stokers, especially if the salt is not replaced in adequate quantities
6. Chronic glomerulonephritis with uremia
7. Diabetes mellitus, especially when acidosis is present
8. Ether anesthesia
9. Lobar pneumonia
10. Occasionally in hepatic necrosis, heart failure, and hypoparathyroidism

POTASSIUM

Our understanding of the physiology and metabolism of potassium has increased tremendously in the past few years. Disease states which are a sequel of excess or deficiency of potassium are now recognized.

Potassium salts are absorbed from the intestinal tract into the bloodstream, and about 90 per cent are excreted in the urine. The distribution of blood potassium is just the opposite of that of sodium, since potassium is much more abundant in the cells. The potassium level in plasma or serum is from 4.1 to 5.6 mEq. per liter, while the concentration in the red blood cells is fifteen to twenty times as great; namely, from 140 to 160 mEq. per liter. Analysis of the whole blood is useless because of variation in the cell content. To obtain accurate results in serum potassium determinations, it is extremely important to prevent hemolysis because of the high potassium content of the red cells.

Potassium, like sodium, helps in the regulation of osmotic pressure and acid-base balance. Its function in these capacities, however, appears to be due to its presence in body tissue and cells, rather than its presence in plasma. The maintenance of potassium-concentration gradient of the red blood cells is dependent on glucose metabolism, and substances which interfere with the latter will also interfere with the maintenance of this gradient.

Less than 10 per cent of the amount of potassium ingested is excreted in the feces, but much larger quantities are lost by this route in cases of diarrhea. Gastric juice contains much higher concentration of potassium than serum, ranging from 15 to 25 mEq. per liter. There is, therefore, considerable loss of potassium in cases of vomiting and gastric suction.

Hyperpotassemia:

An increase in serum potassium is called "hyperpotassemia," or "hyperkalemia," and occurs in the following conditions:

1. Addison's disease. In adrenal cortical insufficiency, the exact mechanism which causes the elevation of serum potassium is not clearly understood. The lack of adrenal cortical hormone causes decreased sodium and increased potassium levels and increased sodium and decreased potassium in the urine. Whether the function of this hormone is to regulate the distribution of body water or whether it is primarily concerned with the regulation of potassium metabolism is still debated.

A significant increase in the serum potassium occurs only during impending or actual crisis.

2. Potassium intake increased beyond the ability of the kidneys to excrete the excess.
3. In oliguria or anuria and uremia. When the kidney damage is caused by breakdown of red cells, not only is the body unable to excrete normal amounts of potassium, but in addition potassium is poured into the extracellular fluid from tissue breakdown. In uremia, the potassium level may be low, normal, or high, depending on the stage of the disease. If renal function is only slightly impaired and the potassium intake is inadequate, or if there is vomiting and/or diarrhea, the serum potassium may be low. If the intake is adequate and renal excretion is not greatly impaired, the potassium level may be normal. When renal function is poor, excretion of potassium is below normal and the serum level is increased.
4. Tissue breakdown which releases potassium from cells into serum.
5. Acute bronchial asthma.
6. Dehydration. This results in a decreased volume of extracellular fluid and a higher level of potassium.

ypopotassemia:

A decrease in serum potassium is called "hypopotassemia," or "hypokalemia," and occurs in the following conditions:

1. During insulin therapy in diabetic acidosis or coma. Prior to the administration of insulin, the serum potassium is usually normal or slightly elevated owing to the reduced extracellular fluid volume caused by dehydration and also to the fact that urinary excretion of potassium does not keep pace with the rapid rate of transfer of potassium from the cells to the serum. During insulin therapy without potassium, there is dilution of the extracellular fluid during administration of fluids, and excessive amounts of potassium are lost in the urine because of diuresis. Further, potassium moves from the serum into the cells under restored carbohydrate metabolism. All these factors cause a drop in the serum potassium level in from four to twenty-four hours.
2. Dilution of the extracellular fluid volume due to the administration of fluids which are free of potassium.
3. Excessive loss of potassium as may occur in Cushing's syndrome.
4. Following administration of large doses of adrenal cortical hormone.
5. Inadequate intake of potassium in the chronically ill and undernourished patients.
6. Loss of potassium in vomiting, gastric suction, diarrhea, and drainage from fistulas.

7. Familial periodic paralysis. This is a complex metabolic disturbance beginning as a weakness in the legs and sometimes spreading to involve other muscles, sometimes all voluntary muscles. An attack lasts from a few hours to several days, and is often nocturnal in onset. Fatigue, exposure to cold, and overeating may precipitate the paralysis, which is always bilateral. The disease is probably intimately associated with carbohydrate metabolism. Administration of potassium abolishes all symptoms, but care should be taken not to raise the patient's potassium level beyond normal.
8. Steatorrhea can increase the loss of potassium in the feces. Unabsorbed intestinal fat combines with various bases including potassium to form insoluble soaps which are excreted. Hypopotassemia, as well as hypocalcemia, may result.
9. Following surgery. It is not uncommon to find a decreased level of serum potassium in patients following surgery. Ingestion of potassium is likely to be low owing to a restricted diet or poor appetite. If vomiting occurs or if gastric suction is started, there is further loss of potassium.

MAGNESIUM

Magnesium is absorbed from the intestinal tract into the bloodstream, and is excreted in the urine and feces. It is present in both the plasma and red blood cells. The level is estimated in the serum, where the normal range is from 1.85 to 2.09 mg. per 100 cc. of blood. Magnesium is increased in the presence of damaged kidneys, especially if magnesium sulfate is taken coincidentally as a cathartic. It is also slightly increased in essential hypertension, arteriosclerosis, and hypertrophic arthritis. It is decreased when there is a low magnesium intake.

CALCIUM

Calcium is absorbed from the upper part of the small intestine. The amount of absorption depends upon the acidity of the intestinal contents and the amount of phosphate present. Calcium salts are soluble in acid but insoluble in alkaline media. In an acid media, therefore, greater amounts of calcium are absorbed. The presence of phosphorus, from a diet high in phosphorus, results in the formation of calcium phosphate, which is only slightly soluble and therefore poorly absorbed. Calcium absorption is also inhibited when there is an excess of fat in the diet, which results in the formation of insoluble calcium soaps.

Calcium is stored chiefly in the bones, from which it is metabolized for physiological needs. It is excreted in the urine and feces.

Blood calcium is found only in the plasma. The technic for determining calcium levels employs serum, in which the normal values range from 9 to 11 mg. per 100 cc. It exists in the blood in two forms, diffusible and nondiffusible. The diffusible calcium exists, for the most part, in the ionized form and represents 55 per cent (5 to 6.5 mg. per 100 cc). The nondiffusible portion is found in serum protein, chiefly the albumin fraction, and represents 45 per cent (4 to 5 mg. per 100 cc.) of the total serum calcium.

Calcium metabolism is influenced by vitamin D, ultraviolet irradiation, and parathyroid extract. It is believed that vitamin D and ultraviolet irradiation increase absorption from the intestinal tract. Vitamin D and parathyroid extract cause hypercalcemia by increasing the withdrawal of calcium from bones. The effect of vitamin D appears, however, to be dependent upon a continued supply of parathyroid hormone. This was confirmed by Taylor and co-workers,[4] who found that toxic changes from overdosages with irradiated ergosterol were less severe than usual in dogs in which complete parathyroidectomies had been performed.

Hypercalcemia:

Hypercalcemia, or increased serum calcium, occurs in the following:

1. Hyperparathyroidism, hyperplasia, or adenoma of the parathyroid glands, which results in increased secretion of parathyroid hormone
2. After excessive dosage of vitamin D
3. Bone tumors, such as multiple myeloma and other neoplastic processes of bone, including metastatic carcinoma
4. Conditions that cause increased carbon-dioxide tension in the blood, which tends to keep calcium in solution, such as emphysema, pneumoconiosis, and cardiac decompensation
5. Erythremia (polycythemia vera)
6. Addison's disease
7. Basophilic adenoma of the pituitary gland
8. Chronic nephritis with uremia

Hypocalcemia:

Hypocalcemia, or decreased serum calcium, occurs in the following:

1. Hypoparathyroidism from disease or following operative removal of parathyroid glands, usually associated with tetany, which is a manifestation of a low calcium level in the blood

2. Vitamin-D deficiency
3. Changes in bone, such as osteomalacia and those due to starvatio with inadequate intake of calcium, phosphorus, and vitami D
4. Conditions resulting in poor absorption of calcium and vitami D, such as sprue and celiac disease
5. Kidney diseases, such as chronic glomerulonephritis with hypertension and increased nitrogen retention, nephrosis, and rena rickets
6. Conditions resulting in decrease in serum albumin, such as systemi leishmaniasis and cachexia from malignancy
7. Obstructive jaundice
8. Late in pregnancy when fetus requires large amounts of calcium and mother does not have an adequate calcium and vitamin-D intake
9. Manic-depressive psychosis

PHOSPHORUS

Phosphates are presumably absorbed in small amounts from the upper portion of the jejunum. They are excreted into the intestine and reabsorbed into the blood from the lower part of the small intestine. The amount of phosphate reabsorbed is in inverse ratio to the amount of calcium excreted into the intestine because it forms an insoluble tertiary calcium phosphate that is not absorbed Absorption and utilization of phosphates are apparently increased by vitamin D. Phosphates are excreted in both the urine and feces, with the greater portions excreted in the feces.

According to Best and Taylor,[5] four phosphorus compounds exist in the blood. One is inorganic phosphorus (orthophosphate) The other fractions are in organic combination as follows:

1. Ester phosphorus, for example glycerophosphates, phosphoglycerates, and hexose phosphates
2. Lipid phosphorus, for example the phosphatides (lecithin cephalin, and sphyngomyelin)
3. Nucleic acid phosphorus

Clinically, the determination of inorganic phosphate in the serum is sufficient. In adults, the normal level is 3.7 mg. per 100 cc., and in children it is 5.6 mg. per 100 cc. Physiological variations occur, with an increase in the summer and a decrease in the winter when the concentration of ultraviolet rays is less. There is also a decrease following ingestion of carbohydrate because of the union of phosphoric acid with carbohydrate to form hexone phosphate.

Hyperphosphatemia:

Hyperphosphatemia, or increased serum-phosphate level, occurs in the following:

1. Hypoparathyroidism
2. Hypervitaminosis (excessive intake of vitamin D)
3. Certain bone diseases, as in multiple myeloma and during healing of fractures
4. Renal disease, such as renal rickets, pyelonephritis, tuberculosis of kidneys, nephritis with edema, nephrosclerosis with nitrogen retention, and polycystic kidneys
5. Addison's disease
6. Acute yellow atrophy of the liver
7. Myelogenous leukemia

Hypophosphatemia:

Hypophosphatemia, or decreased serum-phosphate level, occurs in the following:

1. Hyperparathyroidism with osteitis fibrosa cystica due to increased excretion of phosphate in the urine
2. Osteomalacia
3. Hyperinsulinism, as in adenoma of islands of Langerhans or following administration of insulin
4. Deficient absorption from the intestine:
    - *a.* Vitamin-D deficiency causing rickets, in which there is deficient absorption of both phosphorus and calcium
    - *b.* Tropical and nontropical sprue and celiac disease (excessive amounts of fat in the intestine prevent the absorption of calcium, phosphorus, and vitamin D)
5. Diabetes mellitus
6. After ingestion of carbohydrate or intravenous administration of glucose
7. Normal pregnancy, slight

PHOSPHATASE

Normal serum contains an enzyme or group of enzymes that are capable of liberating inorganic phosphates by hydrolyzing phosphoric esters of erythrocytes and plasma. The most active phosphatase is the phosphatase that requires a *p*H of 9 for optimum activity. For this reason, it is called "alkaline" phosphatase to differentiate it from acid phosphatase, which has rather limited activity in normal serum and an optimum *p*H of 5. Although both enzymes are active at the pH of normal serum, neither has any activity at the optimum pH of the other.

Alkaline Phosphatase:

Hyperphosphatasemia: The normal range for alkaline phosphatase is from 1.5 to 4 Bodansky units per 100 cc. of serum for adults, and from 4 to 14 for children. An increase in phosphatase is called "hyperphosphatasemia," which occurs in the following:

1. Conditions of bones requiring increased deposition of calcium phosphate, such as osteitis fibrosa cystica (Albright's disease) resulting from hyperparathyroidism, osteitis deformans (Paget's disease), osteogensis imperfecta (Lobstein's disease), severe osteomalacia, osteogenic sarcoma, metastatic carcinoma to bones resulting in osteoplastic changes, and familial splenic anemia (Gaucher's disease) with absorption of bone
2. Rickets due to a deficiency of phosphoric ester substrate of erythrocytes
3. Healing fractures
4. Liver disease, such as acute and chronic hepatitis with jaundice, carcinoma of liver with jaundice, and biliary-tract obstruction
5. Renal rickets
6. Myelogenous leukemia
7. Physiologic conditions, such as pregnancy, development of teeth and bones in infancy and childhood, alimentary hyperglycemia, exposure to ultraviolet light, and after administration of irradiated ergosterol

Hypophosphatasemia: Alkaline phosphatase is decreased (hypophosphatasemia) in the following:

1. Cretinism
2. Chronic nephritis, severe
3. Celiac disease

Acid Phosphatase:

Acid phosphatase has optimum activity at pH of 5. The normal range of this enzyme is from 0.5 to 2 Gutman units. Gutman[6] showed that acid phosphatase is formed by carcinoma of the prostate, both at the primary site and in the bone metastases. Carcinoma of the prostate, particularly with bone metastases, causes increased blood acid-phosphatase values as high as 30 units per 100 cc.

AMYLASE

Amylase, also known as "diastase," is a starch-splitting enzyme ound in the blood. The determination of this enzyme is based upon ts ability to split starch into reducing sugar under standard conditions. The normal range is from 70 to 200 mg. of glucose formed by the action of 100 cc. of serum. It is *increased* in the following:

1. Acute pancreatitis
2. Carcinoma of the pancreas
3. Severe renal damage
4. Certain cases of perforated peptic ulcer

It is *decreased* in the following:

1. Necrotizing hepatitis
2. Severe burns with liver damage

LIPASE

Lipase is a fat-splitting enzyme that is found in the blood and is increased under certain conditions. The determination of lipase is based upon its ability to liberate fatty acids from olive oil under standard conditions. The normal range in serum is from 1 to 15 cc. in terms of twentieth-normal sodium hydroxide, that is the amount of twentieth-normal sodium hydroxide required to neutralize the fatty acids formed. It is increased in the following conditions:

1. Acute pancreatitis
2. Carcinoma of the pancreas
3. Cholelithiasis with jaundice
4. Cirrhosis of the liver
5. Carcinoma of the liver
6. Intestinal obstruction
7. Duodenal ulcer

SULFATES

The normal values of inorganic sulfates are from 2.5 to 5 mg. per 100 cc. of serum. They are increased early in all forms of renal damage that cause nitrogen retention:

1. Glomerular nephritis
2. Pyelonephritis
3. Nephrosclerosis with nitrogen retention
4. Polycystic kidneys
5. Obstructions of the urinary tract

IRON

Both organic and inorganic iron is absorbed chiefly from the stomach and duodenum. It is then transported to the liver, where most of it is taken out, and the remainder is stored in the tissues.

Iron is present in the blood in the form of organic iron (hemoglobin) and inorganic iron in the plasma. The organic iron of hemoglobin represents about 52 mg. per 100 cc., or 15.6 gm. of hemoglobin per 100 cc. Inorganic iron in the plasma has a normal range of from 0.8 to 0.17 mg. per 100 cc., which is not of clinical importance. The absorption and utilization of iron are important as far as hemoglobin formation is concerned. Females are apparently unable to absorb iron as well as males, and consequently they are more susceptible to iron-deficiency anemias, represented by hypochromia. Poor absorption or improper metabolism of iron, or both, accounts for hypochromic anemia in both children and adults. Chronic loss of blood also results in hypochromic anemia until the bleeding is stopped and the body is able to bring the hemoglobin of the red cells up to normal.

COPPER

Copper is normally present in the blood in small amounts ranging from 0.14 to 0.15 mg. per 100 cc. It is thought to act as a catalyst in hemoglobin formation, but it does not enter into the structure of the hemoglobin molecule. Lack of an adequate supply of copper appears to be a factor in certain iron-deficiency anemias. Waddell and co-workers[7] found that in young rats, made anemic by being placed upon a diet of whole cow's milk, iron alone failed to promote hemoglobin regeneration. However, when a small amount of copper was added to the diet, blood regeneration was induced.

The determination of the copper level is not clinically important, since anemia due to copper deficiency per se is not known in man.

IODINE

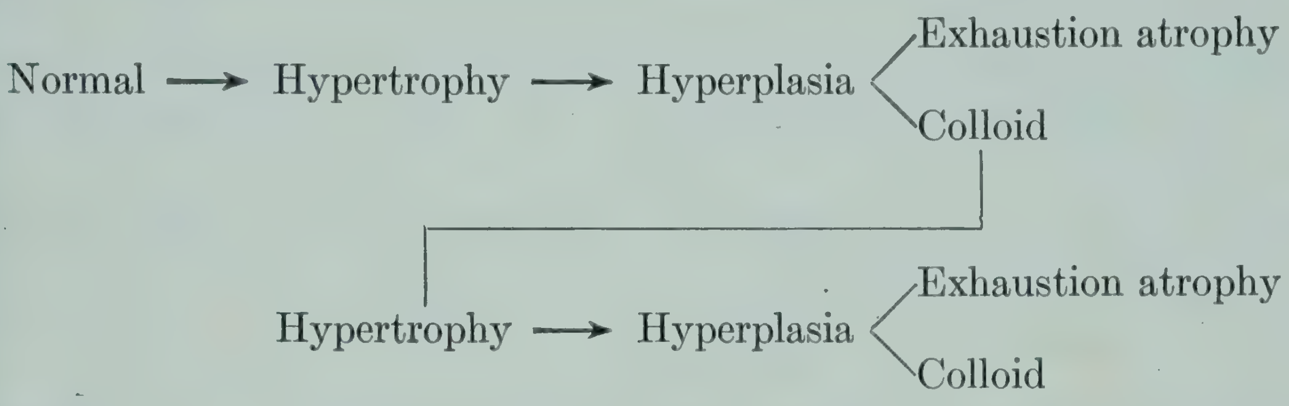
Iodine plays an important role in thyroid hormone formation. Simple goiter is due to iodine deficiency. Best and Taylor[8] indicate the deficiencies as follows:

1. Absolute deficiency in water and food
2. Relative deficiency, iodine intake being sufficient for amount of hormone required under ordinary circumstances of life, but

insufficient under more exacting conditions, such as puberty, pregnancy, excessive protein in diet, and so on

3. Reduced quantity of iodine absorbed from food, owing to microorganisms in intestinal tract

Marine and Williams[9] believe that all thyroid enlargements, ncluding exophthalmic goiter, are examples of "a compensatory vork hypertrophy," brought about by iodine deficiency. The demands made upon the thyroid to produce its hormone without an adequate iodine supply results in hypertrophy, hyperplasia, adenomatous growth, and in some instances final exhaustion atrophy. Marine represents the successive stages as follows:



The normal range of blood iodine is from 10 to 12 micrograms per 100 cc. Increased levels are found in the following:

1. Hyperthyroidism (see Iodine Tolerance Test, p. 223)
2. Jaundice (hepatocellular jaundice and extrahepatic jaundice)
3. Leukemia
4. Physiological states, such as pregnancy and menstruation, after administration of iodine

It is decreased in hypothyroidism, but this is of little or no clinical value, since the diagnosis of this condition is made by other tests.

VITAMIN C

Vitamin C is a relatively simple chemical substance closely allied to hexuronic acid, and it has been named "ascorbic acid" ($C_6H_8O_6$).[9] The chief function of vitamin C appears to be the maintenance, in a normal state, of the intercellular ground substance in which the tissue cells are embedded and cemented together. According to Wolbach,[10] the material is originally liquid but is transformed into a semigelatinous substance, in which the cells are embedded. This gelatin process is dependent upon the action of vitamin C.

The disease caused by a deficiency of vitamin C is scurvy (scorbutus). The essential pathological change in this disease is weakening of the endothelial walls because the intercellular cement substance is reduced in amount. This results in hemorrhages from mucous membranes of the mouth, gastrointestinal tract, skin, subcutaneous tissues, muscles, and subperiosteal tissues.

Scurvy (see p. 537) may be prevented by eating foods that contain vitamin C, such as fruits, fruit juices, and certain vegetables. When the vitamin-C intake is adequate, there is a normal range of ascorbic acid in the blood of from 0.7 to 1.4 mg. per 100 cc. under fasting conditions.

A decrease in ascorbic, or cevitamic, acid in the blood occurs under the following conditions:

1. Scurvy, subclinical or clinical
2. Defective absorption or low intake in gastrointestinal disturbances, rheumatic fever, tuberculosis, liver and kidney damage, and malignancy
3. Increased excretion after administration of acetylsalicylic acid and after ether anesthesia

VITAMIN P

Vitamin P is similar to vitamin C in its location in nature and in the prevention of hemorrhage. It is found particularly in the juice or peel of citrus fruits, paprika, and the leaves of a variety of plants. Certain hemorrhagic diseases with increased capillary permeability have been cured by lemon juice. It is now believed that scurvy is a deficiency disease due to lack of both vitamins C and P. The active principle of vitamin P is hesperidin chalcone. Rutin, which is a flavone glucoside, has a formula similar to hesperidin and acts in a similar manner.

THERAPEUTIC DRUGS

Maintaining an effective level of certain drugs in the blood stream during the course of treatment is often demanded, and this level should be confirmed by chemical examination of the blood. The drugs that require such chemical confirmation are sulfonamides, salicylates, bromide, and thiocyanate.

Sulfonamides:

In treating disease with sulfonamides, it is important to maintain a therapeutic level in the blood. Portions of these compounds are acetylated or conjugated in the liver. The effect of the drug depends upon the amounts escaping acetylation, and it then becomes necessary to know the actual level in the bloodstream. This allows the physician to judge better the amount of dose and frequency of administering the drug. In the usual case, it takes only about eight hours to establish an effective blood level. The following blood levels are recommended for sulfonamides in most infections:

	Mg. Per 100 Cc.
Sulfanilamide	10 to 15
Sulfathiazole	5 to 15
Sulfapyridine	4 to 8
Sulfadiazine	5 to 15

Penicillin:

The determination of body-fluid levels of penicillin is not usually carried out in the routine treatment of patients. Treatment is based upon the efficiency of prescribed dosages. The principle of the procedure for determining the penicillin level is as follows: The blood or other fluid to be analyzed is incubated at various dilutions with a standard inoculum of hemolytic streptococci in the presence of erythrocytes. Dilutions of a standard penicillin solution are treated in the same way. Comparison of the relative dilutions of body fluid and standard required to inhibit the hemolytic action of the test organisms under test conditions shows the penicillin content of the sample.

Investigation by procedures based upon the above principle revealed that the blood level depends upon the size of the dose, method of administration, and the time after dosage when level was determined. Intravenous administration of penicillin is found to drop rapidly to zero. However, with intramuscular administration of the drug, the blood level lasts several hours, although the level is lower. This is the preferred method of administration with repetition of the dose every three hours.

Some penicillin is destroyed within the body, but most of it is excreted by the kidneys.

Streptomycin:

This antibiotic has attracted a great deal of attention because it is relatively less toxic to animals and has greater efficiency against both gram-positive and gram-negative bacteria.

Like penicillin, the determination of the blood or other body-fluid levels of streptomycin is not usually carried out for clinical purposes. The blood level can be determined in the same manner as that for penicillin, but another organism such as Bacillus circulans is used for the bacterial inoculum.

Salicylates:

In the treatment of rheumatic fever with salicylates, Manchester[11] advises that the blood level be kept at least up to 25 mg. per 100 cc. or more. A daily dosage of from 10 to 12 gm. of sodium salicylate is required to maintain this level. Hypoprothrombinemia develops in the early phases of intensive, oral and intravenous, salicylate treatment, according to Manchester. He states further that the prothrombin deficiency improves spontaneously in spite of continued salicylate administration and does not approach a critical level likely to precipitate hemorrhage.

York and Fischer,[12] however, cite a case of a thirteen-year-old boy with a plasma-salicylate level of 64 mg. per 100 cc. and prothrombin activity of less than 10 per cent of normal, who had hematemesis of 600 cc., 500 cc., and 500 cc. on three occasions.

Bromide:

The determination of the blood level of bromide is indicated in bromide poisoning. This may be the result of an overdose by the patient, or it may follow the prolonged use of bromide under the direction of a physician. Sometimes, it is difficult to determine whether certain symptoms are the result of the disease or the result of bromide treatment. In such a case, a determination of the blood level of bromide is valuable to the clinician. The upper limit of safety, in a case under treatment, is considered to be 125 mg. per 100 cc. of blood.

When excessive bromide retention does cause symptoms, it is because it has replaced chloride. When as little as 25 per cent of the chloride is replaced by bromide, toxic symptoms develop. By stopping the drug and administering sodium chloride, the bromide is excreted, and toxic symptoms disappear.

Without bromide medication, there is, according to Wikoff and associates,[13] a normal range of bromine in the serum of from 0.33 to 1.73 mg. per 100 cc.

Thiocyanate:

One of the medical treatments of hypertension is the administration of potassium thiocyanate. Since the drug is toxic at high blood levels, the dosage must be controlled by determination of the blood level periodically. The effective range is from 8 to 12 mg. per 100 cc. of blood, which must be maintained for extended periods. Toxic symptoms sometimes develop with a level of 15 mg. or more, and a level above 35 mg. is a grave risk for the patient.

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CHAPTER 3

Serology

Serological examinations are performed for the detection of antibodies in the serum. They are thought to be globulins or part of the globulin molecule. Antibodies may be natural, or inherited, or they may be acquired as the result of stimulation by an antigen.

ACQUIRED ANTIBODIES

These antibodies develop because of a recognized or unrecognized infection or immunization and are apparently produced by the cells of the reticuloendothelial system. Since there is some delay before the appearance of antibodies, it is preferable to culture the causative organism for early diagnosis.

Antigenicity:

Certain agents, such as spirochetes, viruses, rickettsiae, and bacilli causing typhoid fever, paratyphoid fever, tularemia, and glanders, produce antibodies in large amounts and are designated as highly antigenic. Many gram-positive bacilli, however, including tubercle bacilli, most fungi, and parasites, are poorly antigenic. The laboratory diagnosis of infections by these agents must, therefore, be made by other means.

ANTIBODIES PRODUCED BY ANTIGENIC AGENTS

The antibodies produced by antigenic agents are agglutinins, precipitins, lysins, opsonins, antiviral antibodies, antitoxins, and allergenic antibodies. Although these antibodies produce different reactions, it is thought that they are the same antibody.

Agglutinins:

These are antibodies that cause agglutination, or clumping together, of organisms. Agglutination tests are helpful in the diagnosis of typhoid fever, paratyphoid fever, tularemia, and brucellosis. In these tests, the organisms are suspended in the patient's serum. If specific, agglutinins are present, and the organisms will clump together in groups. If no agglutinins are present, the organisms will remain in homogenous suspension.

Some organisms, such as the typhoid bacillus, produce more than one agglutinin. For example, it produces both somatic and flagellar agglutinins.

Sera containing high titers of specific agglutinins are used for the identification of certain organisms, which are isolated in cultures, such as typhoid and paratyphoid bacilli, and bacilli of the dysentery group. A culture of the unknown organism is made in a liquid medium, and some of this is placed in the specific serum, for example antityphoid serum. If agglutination takes place, the organism is a typhoid bacillus.

Precipitins:

When an antigen is brought into contact with a serum containing specific precipitin antibody, a precipitate forms. In this reaction, the antigen, as well as the antibody, is initially in a state of colloidal solution. Precipitation occurs when any antigen in solution is allowed to react with its corresponding antiserum in the presence of electrolytes, provided that the concentration of each of these reagents and the conditions, such as temperature of incubation, are properly arranged.

An example of the practical application of the precipitin reaction is seen in the test for trichinosis, in which Trichinella-spiralis extract (antigen) is added to serum that contains the specific precipitins.

The current flocculation tests for syphilis are modifications of the precipitin reaction. The syphilitic antibody, or reagin, is not a true precipitin, however, because it produces a flocculation of lipoid particles in colloidal suspension of a nonspecific antigen made of alcoholic extract of beef heart. The flocculation tests for syphilis have largely replaced the more complicated, complement-fixation tests.

Lysins:

Lysins are antibodies that bring about degenerative changes in bacteria or cells. Practically, lysins cannot be differentiated from bactericidins, the antibodies that kill bacteria. Bacteriolysis is not so striking as hemolysis of red cells, although the bacteria undergo a change that is analogous to the lysis of erythrocytes.

The serum under test is inactivated by heat to make ineffective the natural complement. A light suspension of bacteria is added to increasing dilutions of the test serum. A constant amount of bacterial suspension and a constant amount of complement are added. The surviving bacteria in the mixtures are counted, after varying intervals, by a suitable cultural method, and the highest dilution of serum that produces a significant killing effect is noted. According to Topley and Wilson,[1] an important point in connection with bactericidal or bacteriolytic reaction is that its occurrence depends in large part on the nature of the bacterial cell. Certain bacteria, such as the cholera vibrio, the typhoid bacillus, and most gram-negative bacilli, are readily killed and lysed when acted upon by complement after sensitization by a specific antibody. Certain other bacteria, such as gram-positive cocci (staphylococci, and so on), are not susceptible to lysis by antibody and complement.

Opsonins:

In 1903 and 1904, Wright and Douglas[2] described thermolabile substances occurring in normal serum, acting on a variety of bacteria, and rendering them liable to phagocytosis by leukocytes. To these substances, they gave the name "opsonins." The determination of opsonins is used in connection with the vaccine treatment of chronic undulant fever.

Antiviral Antibodies:

Virus disease antibodies are, for the most part, virus neutralizing antibodies. They are similar to antitoxins, but under certain conditions they are virucidal. Virus neutralizing antibodies develop in smallpox, varicella, measles, infantile paralysis, yellow fever, and equine encephalitis.

Although it is not practical for clinical use, virus antibody may be found by using serum and virus neutralization tests upon animals.

Allergenic Antibodies:

Allergin is a type of antibody produced by a foreign protein, causing active or passive sensitization of human beings, and resulting in a state of hypersensitiveness called "allergy."

Antitoxins:

Antitoxins are those antibodies that neutralize toxins. Toxins are highly poisonous substances produced by certain bacterial species, which cause a definite reaction in the host. Some toxins are characterized by the ease with which they may be separated from the bacteria that produce them. Under suitable conditions, they diffuse into a fluid medium and may be obtained from the bacteria by simple filtration. Toxins of this type are referred to as "exotoxins," and they are actively antitoxinogenic. Another class of toxic bacterial products remains incorporated in the bacterial cells. They are referred to as "endotoxins" and are only slightly antitoxinogenic.

We are concerned, at this point, with bacteria that produce exotoxins because they cause the formation of antitoxins. Diphtheria bacilli produce exotoxins, and the body forms neutralizing antitoxins, but not fast enough or in great enough quantities. Consequently, antitoxin must be given therapeutically to prevent damage by the toxins.

The antibodies discussed above are demonstrated in vitro, while antitoxins are demonstrated best in vivo. The Schick and Dick skin tests indicate the presence of antitoxins of diphtheria and scarlet fever. The neutralization of diphtheria toxins by antitoxin in determining the virulence of diphtheria bacilli in guinea pigs is also an in-vivo test.

NATURAL, OR INHERITED, ANTIBODIES

Natural, or inherited, antibodies are found not only in the serum but also in milk, colostrum, and certain transudates and exudates. The only inherited antibodies of practical value are the isohemagglutinins, which will agglutinate red blood cells to determine the blood type. These agglutinins follow the mendelian laws of heredity in the manner of their transmission from parent to child. Although the term "natural resistance" is used, it is difficult to prove the presence of inherited antibodies against specific infections.

BLOOD GROUPS

It is now well known that certain natural antibodies exist in the blood and that they are inherited in accordance with definite genetic laws. The best example of such natural antibodies is the presence of isohemagglutinins, which divide the population into blood groups. In 1901, Landsteiner distinguished the principal blood groups into which all human beings may be divided. He showed that blood groups depend on the distribution of two different antigenic agents, which may be present together, separately, or entirely absent. There are also two corresponding antibodies, which are distributed so that neither antibody is present in the blood of an individual where red blood cells contain the corresponding antigen. By determining the antigens present in any sample of blood, it is possible to place it in the proper group.

Landsteiner indicated the four blood groups by letters as follows: O, A, B, and AB, which represent the antigens of the erythrocytes. This is also known as the "International Nomenclature," and it is used universally at present. Jansky in 1908 and Moss in 1910 in-

TABLE 1: SUMMARY OF ABO SYSTEM

Blood Group	*Isoagglutinins (or Isohemolysins) in Serum*	*Groups of Red Cells Agglutinated (or Hemolyzed) by Antiserum*	*Percentages of Blood Groups (United States)*
O	anti-A and anti-B	A, B, and AB	45
A	anti-B	B and AB	41
B	anti-A	A and AB	10
AB	....	....	4

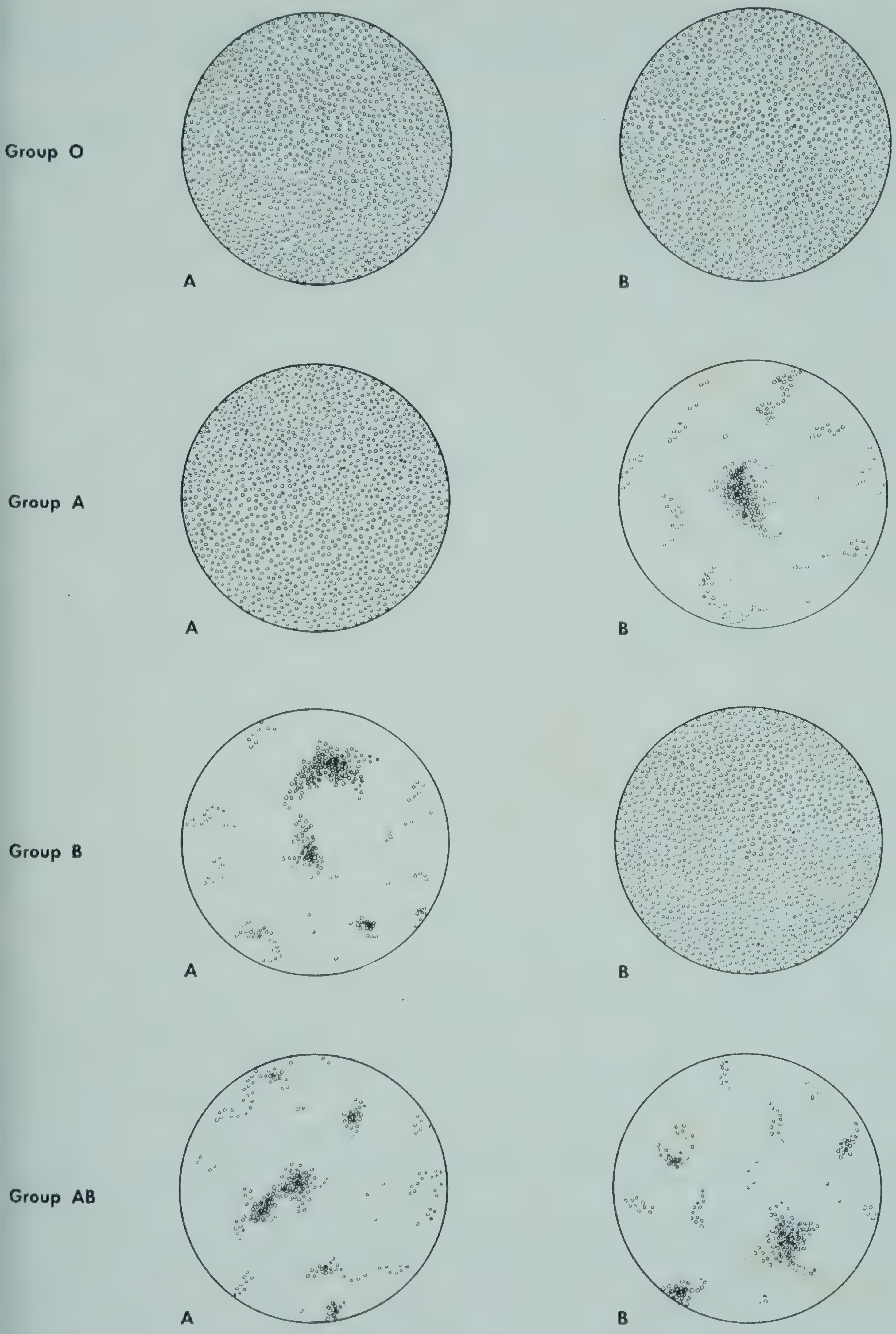
Group O
A
B
Group A
A
B
Group B
A
B
Group AB
A
B

Fig. 1: Blood groups.

dependently assigned numbers to the blood groups, which has caused some confusion because the Group I of Jansky corresponds to Group IV of Moss.

Group O is characterized by the fact that the serum agglutinates the erythrocytes of the other three groups, but the erythrocytes of this group are not agglutinated by any serum.

Group-A serum agglutinates the erythrocytes of B and AB but not those of O and A. The erythrocytes are agglutinated by the serum of groups O or B but not by those of A and AB.

Group-B serum agglutinates the erythrocytes of A and AB but not those of O and B. The erythrocytes are agglutinated by the serum of groups O or A but not by that of B and AB.

Group-AB serum does not agglutinate the erythrocytes of any group. The erythrocytes are agglutinated by the serum of all other groups, that is O, A, and B. Table 2 shows the agglutination of erythrocytes.

TABLE 2: AGGLUTINATION OF ERYTHROCYTES

Erythrocytes	Serum			
	O	A	B	AB
O	–	–	–	–
A	+	–	+	–
B	+	+	–	–
AB	+	+	+	–

+ = agglutination and – = no agglutination

The grouping of unknown bloods is illustrated in Table 3. Only sera of groups A and B are necessary for grouping unknown bloods.

TABLE 3: GROUPING OF UNKNOWN BLOODS

Cells of Unknown Blood	Reaction with Group A (Anti-B) serum	Reaction with Group B (Anti-A) serum	Blood Group
X	–	–	O
X	–	+	A
X	+	–	B
X	+	+	AB

+ = agglutination and – = no agglutination

Subgroups of A:

Groups A and AB can each be subdivided owing to the existence of two major varieties of agglutinogen A, A_1, and A_2.* Thus, group A is subdivided into subgroups A_1 and A_2; and group AB into subgroups A_1B and A_2B. Agglutinins specific for the subgroups of A rarely occur in normal serum, although they may be produced by repeated blood transfusions of one of these subgroups. These same subgroup agglutinins may be produced in a mother during pregnancy by isoimmunization by the blood of the fetus.

THE RH FACTOR

Landsteiner and Wiener[3] discovered the presence of another agglutinogen in human blood to which they gave the name "Rh" from the first two letters of rhesus, the monkey used in the experiments. When rabbits were given injections of blood from Macacus rhesus monkeys, they produced antibodies that would agglutinate the blood cells of all rhesus monkeys and of about 85 per cent of the white population of the United States. The cells that could be agglutinated by serum containing anti-Rh were called "Rh-positive" (Rh+); and those that the serum could not agglutinate were called "Rh-negative" (Rh—). The discovery of the Rh factor has proved to be of great help in explaining certain transfusion reactions and the pathogenesis of congenital hemolytic disease (erythroblastosis foetalis) (see also p. 335).

Further investigation by Wiener[4] showed that there are three distinct varieties of Rh antiserums obtainable from human sources. The one corresponding to the original antirhesus serum, reacting with the blood of approximately 85 per cent of Caucasians, has been designated[5] as "anti-Rh_0." The other two antiserums were designated "anti-rh′," for the one giving 70 per cent positive reactions,[6] and "anti-rh″," for the one giving 30 per cent reactions.[4,7] These three varieties of Rh antiserums detect the presence or absence of three corresponding Rh factors in human blood: Rh_0, rh′, and rh″. Anti-Rh_0 serum is the most important, and its use will solve most clinical problems.

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* Other rare variants of A are designated as A_3, A_4, and so on.

When all three antiserums are used for testing the Rh factor, human bloods can be subdivided into eight types.[8] Bloods containing the Rh_0 factor (Rh-positive blood) may fall into any one of four types, and bloods not containing the Rh_0 factor (Rh-negative blood) also have four types. These are shown in Table 4.

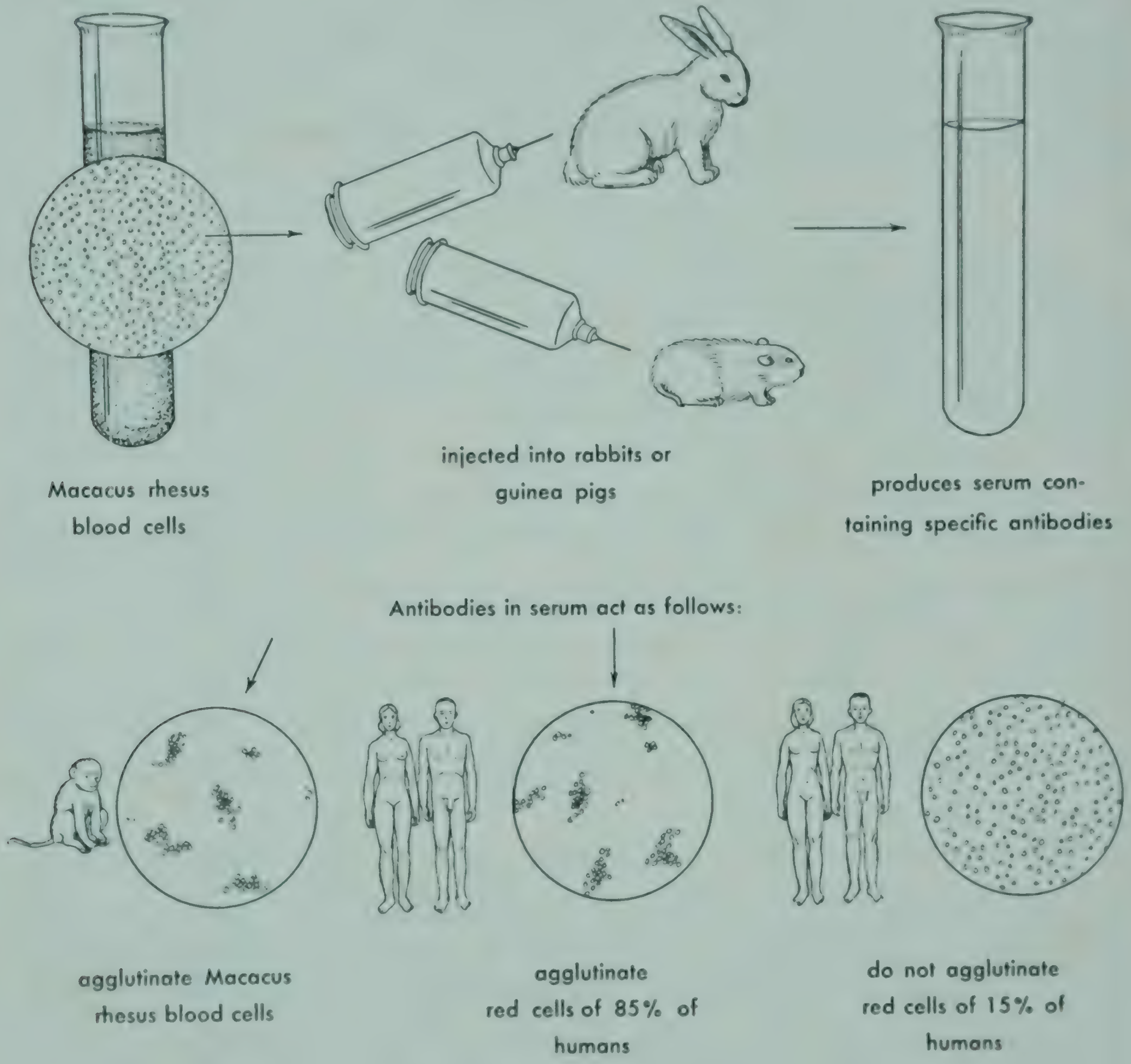
Macacus rhesus blood cells
injected into rabbits or guinea pigs
produces serum containing specific antibodies
Antibodies in serum act as follows:
agglutinate Macacus rhesus blood cells
agglutinate red cells of 85% of humans
do not agglutinate red cells of 15% of humans

Fig. 2: Rh antibodies.

Anti-Hr Factor:

Another antibody with a specificity different from that of any of the known Rh agglutinins was that reported by Levine and co-workers.[9] It agglutinated all Rh-negative cells and many Rh-positive cells; and since it apparently had some relation to Rh, these letters were reversed and it was called “anti-Hr.” Other antibodies of a similar nature were reported by Race and Taylor[10] under the name of “anti-St.” These antibodies react with all bloods from individuals failing to possess two genes determining the presence of rh′, and for this reason they have been called “anti-hr′ agglutinins.” The antigen that produces them is called “hr′.” The use of anti-hr′ plus the three types of anti-Rh serum (anti-rh′, and anti-rh″, and anti-Rh_0) makes it theoretically possible to determine ten types of blood, of which two are very rare.

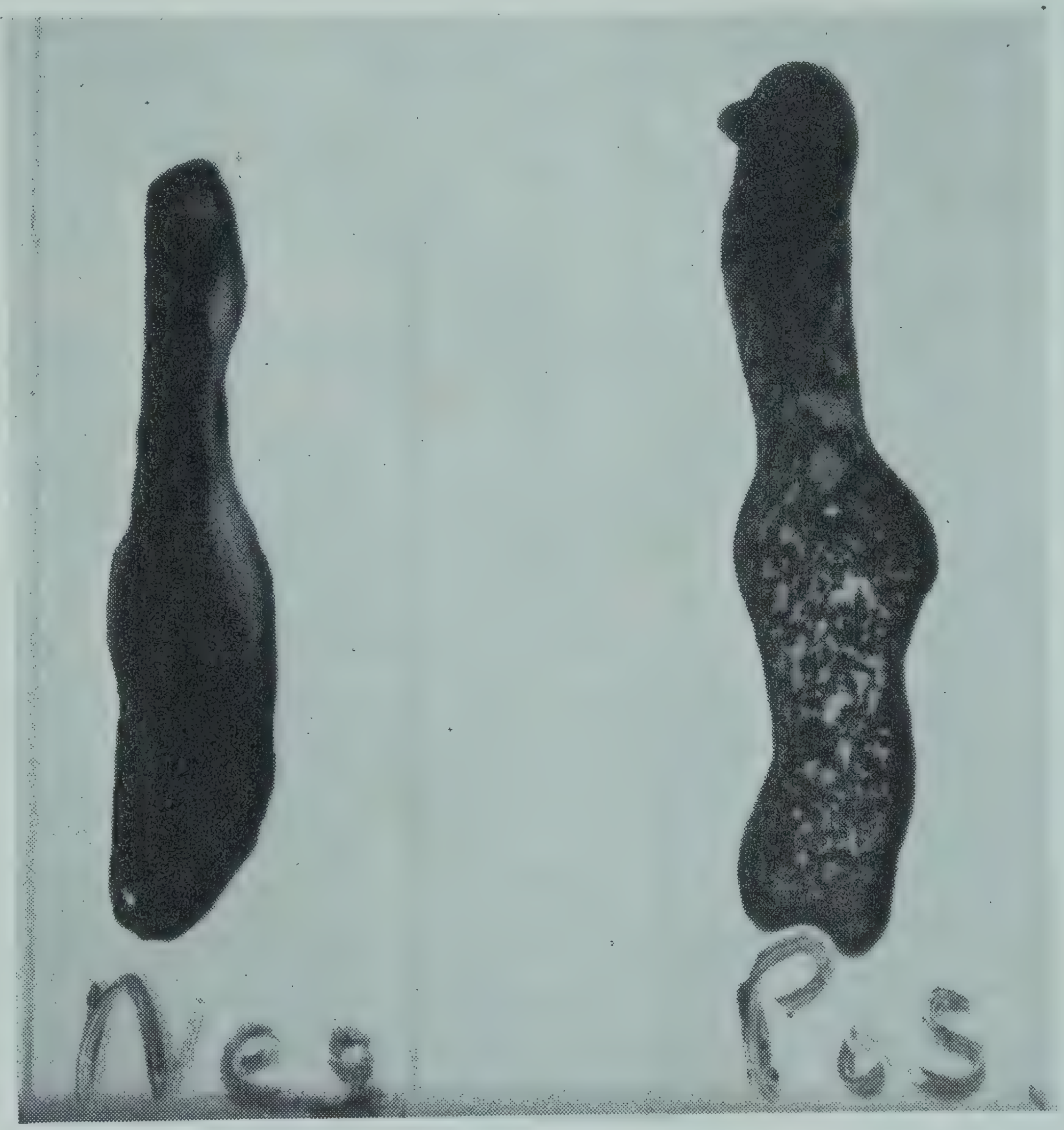

Fig. 3: Slide test demonstrating negative and positive Rh bloods.

Another variety of anti-Hr serum was discovered by Mourant.[11] This serum reacts with an antigen that is present in all cells except those possessing two genes responsible for the presence of rh″. Since these antibodies react with an antigen present only when at least one rh″ gene is lacking, they are called “anti-hr″ agglutinins.” The name “hr″” is given to the corresponding antigen.

TABLE 4: SCHEME OF THE EIGHT RH BLOOD TYPES

BLOOD CONTAINING FACTOR RH_0 (Rh Positive)				BLOOD NOT CONTAINING FACTOR RH_0 (Rh Negative)			
*Designation of Types**	*Reactions with Sera*			*Designation of Types*	*Reactions with Sera*		
	Anti- rh′	*Anti-* rh″	*Anti-* Rh_0		*Anti-* rh′	*Anti-* rh″	*Anti-* Rh_0
Rh_0(D)	−	−	+	rh (cde)	−	−	−
Rh_1 (CD)	+	−	+	rh′ (C)	+	−	−
Rh_2 (DE)	−	+	+	rh″ (E)	−	+	−
$\mathrm{Rh}_1\mathrm{Rh}_2$ (CDE)	+	+	+	rh′rh″ (CE)	+	+	−

* Type Rh_1 contains two factors Rh_0 and rh′, the name "Rh_1" being short for "Rh'_0"; similarly, "Rh_2" is short for "Rh''_0," and "$\mathrm{Rh}_1\mathrm{Rh}_2$" is short for "$\mathrm{Rh}'_0\mathrm{Rh}''_0$."

In 1946, Diamond[12] reported an anti-Hr serum that had a specificity reciprocal to anti-Rh_0. This completed the series of Hr antibodies that Fisher[13] had postulated. If these six serums were available, twenty-seven types of Rh-Hr blood could be identified.

Since the Hr factors are much less antigenic than Rh factors, they rarely cause intragroup transfusion reactions or erythroblastosis foetalis. According to Wiener and Sonn,[14] they have been used as a presumptive test for homozygosity and heterozygosity for those who are type Rh_1 or type Rh_2, particularly in determining the prognosis for future pregnancies in cases where females have become sensitized to the Rh factor. They are also useful in cases of disputed parentage.[8]

CDE Nomenclature:

There has been much confusion among both physicians and laity because of the complex terminology and the rapid progress in this field of serology. The confusion can be eliminated by employing the nomenclature of Fisher and Race, which is based on assigning letters (C, D, E, c, d, e) to the specific antigens of the Rh system.

It has been recommended that the CDE nomenclature be adopted as standard, but the Rh terminology of Landsteiner and Wiener must also be understood for one to converse and read intelligently on the subject. Table 4 gives both nomenclatures in brief.

TABLE 5: THE RH SYSTEM*[46]

Frequency of Subgroups and Genotypes for British Population					*Agglutination by Anti-sera (Per Cent)*					
Subgroups		*Genotypes*			Anti-C (anti-Rh')–70	Anti-D (anti-Rh_0)–85	Anti-E (anti-Rh")–30	Anti-c (anti-Hr')–80	Anti-d (anti-Hr_0)–63	Anti-e (anti-Hr")–97
Wiener Nomenclature	*Frequency (Per Cent)*	*Fisher-Race*	*Wiener Modified*	*Calculated Frequency (Per Cent)*						
rh	15.1	cde/cde	rr	15.1020	O	O	O	+	+	+
rh'	0.8	Cde/Cde	R'R'	0.0097	+	O	O	O	+	+
		Cde/cde	R'r	.7644	+	O	O	+	+	+
Rh_0	2.1	cDe/cDe	R_0R_0	0.0659	O	+	O	+	O	+
		cDe/cde	R_0r	1.9950	O	+	O	+	+	+
rh"	0.9	cdE/cdE	R"R"	0.0141	O	O	+	+	+	O
		cdE/cde	R"r	.9235	O	O	+	+	+	+
Rh_1	53.4	CDe/CDe	R_1R_1	17.6803	+	+	O	O	O	+
		CDe/Cde	R_1R'	0.8270	+	+	O	O	+	+
		CDe/cDe	R_1R_0	2.1585	+	+	O	+	O	+
		CDe/cde	R_1r	32.6808	+	+	O	+	+	+
		Cde/cDe	$R'R_0$	0.0505	+	+	O	+	+	+
Rh_2	14.1	cDE/cDE	R_2R_2	1.9906	O	+	+	+	O	O
		cDE/cDe	R_2R_0	0.7243	O	+	+	+	O	+
		cDE/cdE	R_2R''	.3353	O	+	+	+	+	O
		cDE/cde	R_2r	10.9657	O	+	+	+	+	+
		cDe/cdE	R_0R''	0.0610	O	+	+	+	+	+
rh'rh"	0.03	CdE/CdE	R_YR_Y	0.0000003	+	O	+	O	+	O
		CdE/Cde	R_YR'	.0001	+	O	+	O	+	+
		CdE/cdE	R_YR''	.0001	+	O	+	+	+	O
		CdE/cde	R_Yr	.0039	+	O	+	+	+	+
		Cde/cdE	R'R"	.0234	+	O	+	+	+	+
Rh_1Rh_2	13.6	CDE/CDE	R_ZR_Z	0.0006	+	+	+	O	O	O
		CDE/CDe	R_ZR_1	.2048	+	+	+	O	O	+
		CDE/cDE	R_ZR_2	.0687	+	+	+	+	O	O
		CDE/CdE	R_ZR_Y	.00002	+	+	+	O	+	O
		CDE/Cde	R_ZR'	.0048	+	+	+	O	+	+
		CDe/CdE	R_1R_Y	.0042	+	+	+	O	+	+
		CDE/cDe	R_ZR_0	.0125	+	+	+	+	O	+
		CDe/cDE	R_1R_2	11.8648	+	+	+	+	O	+
		CDE/cdE	R_ZR''	0.0058	+	+	+	+	+	O
		cDE/CdE	R_2R_Y	.0014	+	+	+	+	+	O
		CDE/cde	R_Zr	.1893	+	+	+	+	+	+
		CDe/cdE	R_1R''	.9992	+	+	+	+	+	+
		cDE/Cde	R_2R'	.2775	+	+	+	+	+	+
		CdE/cDe	R_YR_0	.0003	+	+	+	+	+	+

*Race, R. R., Mourant, A. E., Lawler, S. D., and Sanger, R.: The Rh Chromosome Frequencies in England. J. Hematol., 3: 689, 1948—a study of 2000 blood samples. The frequency of the R_Y gene is taken as 0.00005; see Fisher, R. A., and Race, R. R.: Rh Gene Frequency in England. Nature, 157: 48, 1946. The frequencies are approximately the same for American whites, the proportion of D-negative being a little lower (approximately 15 per cent).

Transfusion Reactions:

When a susceptible Rh-negative person is transfused with Rh-positive cells, antibody formation may be initiated. If several transfusions are given a few weeks apart, antibodies may be produced in such great concentration that, when Rh-positive cells are introduced into the circulation at a later date, hemolysis may take place and may be the cause of so-called "transfusion reaction." Some of these reactions may result in death. Because of this danger, all Rh-negative persons requiring transfusions should receive Rh-negative blood. The Rh status of recipients and donors should be determined so that homologous Rh-factor blood may be given as well as the homologous blood group.

The administration of Rh-positive blood to an Rh-negative female patient may stimulate the formation of antibodies and may make it impossible for her to bear normal children subsequently if her husband is Rh-positive. Giving Rh-positive blood to an Rh-negative woman before she has become pregnant may be responsible for erythroblastosis foetalis in her offspring.

Mothers of children with hemolytic disease usually have severe reactions to Rh-positive blood. No Rh-negative woman who has delivered an infant with hemolytic disease should be given Rh-positive blood. Furthermore, no woman whose offspring died in the first few days of life or before birth from unknown causes should be given Rh-positive blood unless she is known to be Rh-positive or can be shown to be free of Rh antibodies. In this way, certain harmful transfusion reactions can be avoided.

Rh Factor and Erythroblastosis Foetalis:

Congenital hemolytic disease (see also p. 335) is caused by Rh antibodies in the mother's blood. An Rh-negative woman married to an Rh-positive man can expect all of her children to be Rh-positive if her husband is homozygous, and half to be Rh-positive if he is heterozygous. Apparently during pregnancy with the above Rh combination of parents, the blood of an Rh-positive fetus escapes into the maternal circulation. Antibodies may be produced by the mother against these cells. These antibodies filter readily through the placenta into the fetal circulation and thus may act upon the erythrocytes of the fetus and destroy them. This process results in erythroblastosis foetalis, or congenital hemolytic disease. The first child is rarely affected; but with increasing concentration of antibodies, subsequent children may develop the disease.

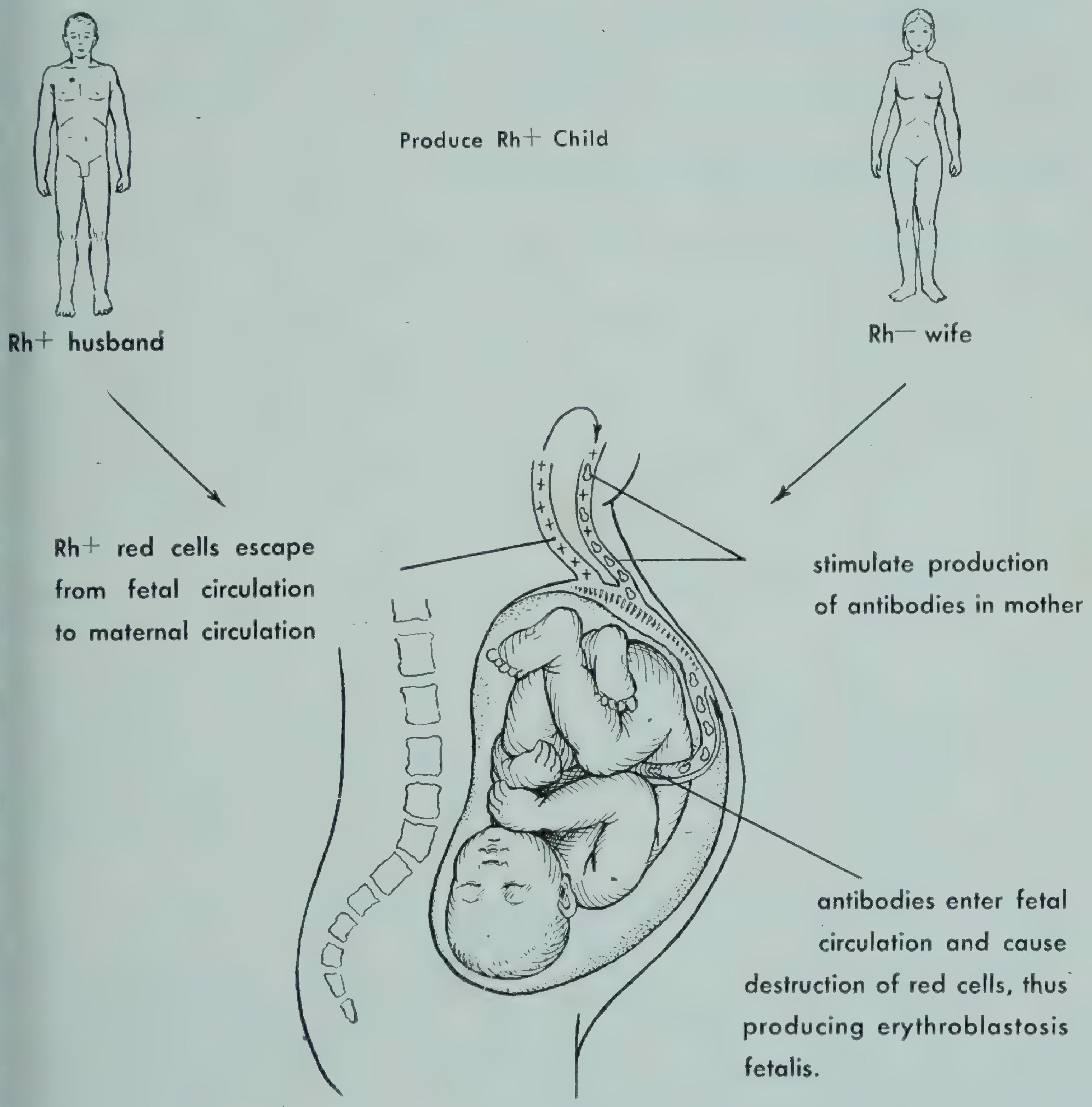
Produce Rh+ Child
Rh+ husband
Rh− wife
Rh+ red cells escape from fetal circulation to maternal circulation
stimulate production of antibodies in mother
antibodies enter fetal circulation and cause destruction of red cells, thus producing erythroblastosis fetalis.

Fig. 4: Development of erythroblastosis foetalis.

False Rh-Negative Donors: All Rh-negative (D-negative) donors by direct typing should be tested for the D^u factor by the indirect Coombs test. This will reveal a certain number of Rh-positive donors who were falsely Rh-negative by the direct test, and will decrease further the number of transfusion reactions.

Antenatal Examination: It is necessary to determine the husband's Rh factor in all pregnancies of Rh-negative women. If the husband is Rh-positive, the wife's blood should be examined for Rh antibodies each month of the last trimester. If the blood shows an Rh-antibody titer, all precautions should be taken to treat the infant as a case of congenital hemolytic disease until proved otherwise.

Blood Groups in Establishing Parentage:

According to Wiener,[15] blood grouping may be applied in medicolegal investigation in the following types of cases:

1. A child is born in lawful wedlock and the husband denies paternity. The law presumes that all children born in lawful wedlock are legitimate (in order to protect the interests of the child) unless it is proved that it is impossible for the husband to be the father of the child. As proof of nonpaternity, the courts permit the introduction of evidence of nonaccess and of impotence or sterility of the husband.
2. A child is born out of lawful wedlock, and the man named by the mother as its father denies paternity.
3. Two newborn infants have been accidentally interchanged in a hospital shortly after birth; and it is desired to identify the parents of the infants, or it is suspected that a wetnurse has wilfully substituted her own infant for the one placed in her custody so that her own child may have the benefits of a better home.
4. A woman has simulated pregnancy and childbirth and now pretends that a certain child is her own in order to compel a man to marry her, to obtain dower rights in her dead husband's estate (in jurisdictions where birth of issue is a prerequisite to the right of dower), or in order that the child may become the heir to her husband's estate.

It must be made clear that the value of blood groups lies in proving *nonpaternity*. Determination of blood groups cannot prove that a certain man is the father of a child because any man with the same blood group could be the father. The chief value, therefore, lies in excluding certain men who may have been accused falsely.

Agglutinogens A, B, M, and N are inherited according to definite laws. By using all four agglutinogens, one out of three falsely accused males may be exonerated. The possible blood groups of children from combinations of parents of known major blood groups are shown in Table 6.

TABLE 6: LANDSTEINER BLOOD GROUPS IN PARENTS AND CHILDREN*

Groups of Parents	*Groups of Children Possible*	*Groups of Children not Possible*
1. O + O	O	A, B, AB
2. O + A	O, A	B, AB
3. O + B	O, B	A, AB
4. A + A	O, A	B, AB
5. A + B	O, A, B, AB	O
6. B + B	O, B	A, AB
7. O + AB	A, B	O, AB
8. A + AB	A, B, AB	O
9. B + AB	A, B, AB	O
10. AB + AB	A, B, AB	O

* Wiener, A. S.: Blood Groups and Transfusion. Charles C. Thomas, Publisher, Springfield, Ill., 1946, p. 381.

In cases where a decision cannot be reached from the major blood groups, examination for M and N types should be carried out. The types of children from the MN parent combination are shown in Table 7.

TABLE 7: AGGLUTINOGENS M AND N IN PARENTS AND CHILDREN*

Types of Parents	*Types of Children Possible*	*Types of Children not Possible*
1. MN + MN	M, N, and MN	O
2. MN + N	N and MN	M
3. MN + M	M and MN	N
4. M + N	MN	M and N
5. N + N	N	M and MN
6. M + M	M	N and MN

* Wiener, A. S.: Blood Groups and Transfusion. Charles C. Thomas, Publisher, Springfield, Ill., 1946, p. 386.

Laws of Heredity: The following laws of heredity compiled by Wiener[8,15] are helpful in the determination of nonpaternity:

1. The blood groups (O, A, B, and AB):
    - *a.* The agglutinogens A and B cannot appear in the blood of a child unless present in the blood of one or both parents.
    - *b.* Group-AB parents cannot have group-O children, and group-O parents cannot have group-AB children.
2. The blood types (M, N, and MN):
    - *a.* The agglutinogens M and N cannot appear in the blood of a child unless present in the blood of one or both parents.
    - *b.* Type-M parents cannot have type-N children, and type-N parents cannot have type-M children.
3. The subgroups of group A (A_1 and A_2) and group AB (A_1B and A_2B):
    - *a.* No child can belong to subgroup A_1 or subgroup A_1B unless one or both parents belongs to one of these subgroups. For example, two parents both of subgroup A_2 cannot have a child of subgroup A_1.
    - *b.* Parents of subgroup A_1B cannot have children of subgroup A_2, and parents of subgroup A_2 cannot have children of subgroup A_1B.

    Note: When a man is excluded by these tests, it is highly probable, though not absolutely certain, that he is not the father of the child in question.
4. The Rh blood types [rh, rh′, rh″, rh′rh″, Rh_0, Rh_1, (or Rh_0'), Rh_2 (or Rh_0''), and Rh_1Rh_2]:

    The Hr Factors: $Rh_1Rh_1 = Rh_1 hr'—$, $Rh_1rh = Rh_1\ hr'+$
    $Rh_2Rh_2 = Rh_2\ hr''—$, $Rh_2rh = Rh_2\ hr''+$
    $rh'rh' = rh'\ hr'—$, $rh'rh = rh'\ hr+$
    $rh''rh'' = rh''\ hr''—$, $rh''rh = rh''\ hr''+$

    - *a.* Factors Rh_0, rh′, rh″, hr′, and hr″ cannot appear in the blood of a child unless present in the blood of one or both parents.
    - *b.* Parents of type Rh_1Rh_1 and rh′rh′ cannot have children of types rh, Rh_0, Rh_2, or rh″, and parents of types rh, Rh_0, Rh_2, and rh″ cannot have children of types Rh_1Rh_1 or rh′rh′.
    - *c.* Parents of types Rh_2Rh_2 and rh″rh″ cannot have children of types rh, Rh_0, Rh_1, or rh′ and parents of types rh, Rh_0, Rh_1, and rh′ cannot have children of types Rh_2Rh_2 or rh″rh″.

COLD AGGLUTININS

Autoagglutinins are present in certain people and are of the "cold agglutinin" variety because they may agglutinate or hemolyze the individual's erythrocytes when the temperature is reduced to 32° to 41° F. (0 to 5° C.). Such autoagglutinins are thought to be present in virus pneumonia, paroxysmal hemoglobinemia, hemolytic jaundice, Raynaud's disease, and certain streptococcus and staphylococcus infections.

NONSPECIFIC ANTIBODIES

Some organisms, such as typhoid bacilli, produce group antibodies, which, in addition to homologous agglutination, cause cross-agglutination of paratyphoid bacilli. Therefore, the specificity of the antibodies for the particular disease must be based upon the height of the titer.

The rickettsial organisms, which cause typhus and Rocky Mountain spotted fever, produce a nonspecific agglutinin that for some reason causes agglutination of certain strains of Proteus vulgaris. This is the so-called "Weil-Felix reaction," which is a very helpful procedure in the laboratory diagnosis of these two rickettsial diseases. Thus, rickettsias and the Proteus vulgaris apparently have a common antigenic feature wherein rickettsial antibodies can agglutinate an unrelated saprophytic bacillus such as Proteus vulgaris.

Another type of nonspecific antibody is the so-called "heterophile antibody" that is present in small amounts in human serum. This antibody is an antisheep cell agglutinin and hemolysin. Normal human serum agglutinates sheep cells in low dilutions. The titer is increased in infectious mononucleosis so constantly that it is now used as a laboratory aid in diagnosing this disease.

BACTERIAL AGGLUTINATION TESTS

INFECTION WITH TYPHOID BACILLUS

The Widal agglutination test has been widely employed in the diagnosis of typhoid fever. Agglutinins do not develop until after the first week of the disease. During the first week, a blood culture is the best diagnostic procedure. The microscopic Widal test has been largely replaced by macroscopic tests in which somatic and flagellar antigens are used.

Since most of the organisms of the salmonella group are flagellated, both the H (flagellar) and O (somatic) antigens must be considered. A formalized broth culture of a flagellated organism agglutinates readily in the presence of homologous H agglutinins but responds poorly, if at all, to homologous O agglutinins. This may be explained by the fact that formalin fixes the flagella over the bacterial surface in such a way that somatic (O) antigens are no longer exposed to the action of the O agglutinins. In testing for the O antigens, a bacterial suspension is extracted with hot alcohol, thus inactivating the flagellar antigen.

Vi Antigen:

Felix and Pitt[16] showed that the virulence of certain strains of typhoid bacilli and their agglutinability by an O antiserum were due to the presence of a special antigenic component, which Felix chose to call the "Vi antigen." This antigen is heat labile; therefore agglutination tests to determine its presence must be carried out with living organisms at 98.6° F. (37° C.) . It was noted that rabbits immunized with living cultures of highly virulent typhoid bacilli produced, in addition to the usual O (somatic) antibodies, a Vi antibody which agglutinated the virulent strains. On the other hand, rabbits immunized with avirulent smooth strains produced only O antibodies. A Vi antiserum gives effective passive protection in mice against virulent strains of typhoid bacilli, while O antiserum provides little or no protection.

Active Typhoid Fever:

In evaluating the results of typhoid agglutination tests, the possibility of previous immunization must be considered. Agglutination of H antigen at 1:80 or higher is interpreted as indicative of the following: (1) active typhoid fever, (2) previous immunization against typhoid fever, or (3) a previous attack of typhoid fever. If the O antigen shows a titer of 1:160 or higher, it usually means that the person has typhoid fever. A rising titer is especially significant in relation to active disease.

Previous Immunization Against Typhoid Fever:

Active immunization against typhoid fever with typhoid-paratyphoid vaccine produces a high titer of H agglutinins and a lower titer of O agglutinins.[17] If an immunized person is ill and has a high titer of H agglutinins and a low titer of O agglutinins, the infection is probably due to an organism other than the typhoid bacillus. On the other hand, an exceedingly high H-agglutinin titer above 1:2000 is suggestive of typhoid fever because H-agglutinin titers above 1:640 do not last for more than five or six months after immunization.

Typhoid Carriers:

Typhoid carriers are best identified by culturing the stools. However, not all persons who have recovered from typhoid fever are carriers. In evaluating a positive titer, the possibility of a previous attack of typhoid fever must be considered. A carrier almost always shows H or O agglutinins or both, usually in low titer. It was suggested that agglutination of Vi antigen would be of help in detecting carriers;[18,19] but in view of the low titers observed by Horgan and Drysdale,[20] its use in detecting carriers was not advised.

INFECTION WITH PARATYPHOID BACILLI

Paratyphoid fever is caused by Bacillus paratyphosus A (Salmonella paratyphi) and Bacillus paratyphosus B (Salmonella schottmülleri). The diagnosis of this disease is made preferably by culturing the causative organism from the feces because the agglutination titer is not always significant, particularly on only one examination. Macroscopic agglutination tests using both O and H antigens of both organisms are preferable. Normal persons will agglutinate the H antigen up to 1:20 and the O antigen up to 1:80. Agglutination of the H antigen at 1:80, or higher, and the O antigen at 1:60, or higher, is significant. A rising titer of both antigens is especially significant. In persons who have been immunized against paratyphoid fever, the active disease is diagnosed only after progressive increase of the O antigen of either causative organism.

There is some increase in agglutinins for typhoid bacilli during paratyphoid fever. This so-called "crossagglutination" is thought to be due to the close relationship between the two groups of organisms.

INFECTION WITH BRUCELLA ORGANISMS

The diagnosis of brucellosis is best made by culturing the organisms. Positive cultures, however, are rarely obtained; and the process of culture and identification is so long that an agglutination test, skin test, and opsonocytophagic index are used in an attempt to make the diagnosis.

Most infections appear to be caused by Brucella abortus, but it is advisable to employ both Brucella abortus and Brucella melitensis strains in doing agglutination tests. According to Menefee and Poston,[21] normal persons have either negative agglutination tests or are positive in low dilutions, usually not over 1:10. Positive agglutination tests of 1:40, or higher, are of significance, but they must be carefully evaluated in relation to the clinical symptoms. In fact, to make a diagnosis on agglutination tests alone, a rising titer in the presence of the clinical syndrome is preferable. Unfortunately, a negative agglutination test does not rule out brucellosis because the occurrence of agglutinins is unpredictable. The agglutination test may remain negative in the presence of a positive blood culture. On the other hand, persons who have had raw milk for years may show a higher titer than 1:10 and may have no clinical symptoms of the disease at the time of examination.

The intradermal skin test is usually employed in conjunction with the agglutination test and opsonocytophagic index. The agglutination test and opsonocytophagic index should be done before the skin test because the introduction of antigen may produce agglutinins and opsonins. A negative skin test appears to rule out brucellar infection both past and present. The skin test, on the other hand, may be positive in the absence of acute or chronic infection because of the production of antibodies by previous subclinical infection.

INFECTION WITH PASTEURELLA TULARENSIS

Since it is difficult to isolate the causative organism of tularemia, immunological diagnostic procedures are employed to make a presumptive diagnosis. Agglutinins for Pasteurella tularensis begin to appear in the second week of illness and reach a maximum titer during the second month. A titer of 1:40 is considered significant, and a maximum titer may reach 1:2560.

It has been noted that crossagglutination with Brucellae melitenses and abortus organisms, and Proteus vulgaris OX_{19} occurs in 25 per cent of cases. In some cases of brucellosis,[22] there is also a crossagglutination with Pasteurella tularensis organisms.

According to Foshay,[23] skin testing with heat-killed, detoxified bacteria gives a positive diagnosis earlier (third or fourth day) in the course of the disease. The skin test is performed by injecting 0.01 cc. of material (one billion organisms per cubic centimeter) intradermally. A positive reaction with edema and erythema begins to develop in thirty-six hours. This test apparently is very specific for Pasteurella tularensis but usually persists for years after the acute infection. Consequently, a positive test must be correlated with the current clinical symptoms because it may be the result of tularemia some time previously. It is important to use detoxified and diluted organisms because ordinary, heat-killed bacteria in a heavy concentration will cause necrosis.

Foshay[24] found that patients who react positively to the bacterial skin test with a delayed reaction, as described above, give an immediate transient reaction to an intradermal injection of his antitularemic serum. To rule out a reaction to serum protein, normal serum from the same animal should be injected. A third test of serum from the same animal immunized with another organism is also highly desirable.

Opsonocytophagic Index:

A formalin-killed suspension of Pasteurella tularensis is used to perform this test, following a technic similar to that used for brucellosis. The test is usually positive about the third week of the disease. According to Friedewald and Hunt,[25] it is difficult to interpret because positive tests have been found in conditions other than tularemia.

LEPTOSPIROSIS (WEIL'S DISEASE)

This is an infection of human beings most commonly caused by Leptospira icterohemorrhagiae. Leptospirosis also occurs as a result of infection by Leptospira canicola, Leptospira grippotyphosa, or Leptospira hebdomidis. In the United States, practically all infections have been caused by Leptospirae icterohemorrhagiae and canicolae.

It is preferable to make the diagnosis by identifying the spirochetes in the blood, by darkfield examination, or by inoculating guinea pigs with blood and urine. Agglutination tests, however, are highly specific, even though normal persons will agglutinate the spirochetes in low dilutions up to 1:40. During the disease, the titer increases from the fifth day on so that, by the end of the second week, the titer may reach 1:100,000. According to Fairley,[26] agglutinins continue in high titer for about seven weeks; and after recovery of the patient, they decrease to 1:30 to 1:900 and are present for years.

Antigenically, Leptospira icterohemorrhagiae appears to differ from other leptospiral strains that are pathogenic for human beings. It would seem advisable, therefore, to use homologous species of leptospira in performing agglutination tests.

Some cases of leptospirosis are said to develop transient, positive, Wassermann reactions.

BACTERIOLOGICAL EXAMINATION OF BLOOD

This subject is discussed in Chapter 13 (p. 253).

NONSPECIFIC AGGLUTINATION TESTS

Infectious Mononucleosis

The causative agent of this disease has not been isolated, but some believe it is caused by a virus.

In 1932, Paul and Bunnell[27] reported that sera of patients with infectious mononucleosis (see also p. 379) commonly show an increased titer of heterophile agglutinins for washed sheep cells. The test has proved to be quite constant and characteristic. For these reasons, it is of real value in establishing a diagnosis, particularly if there is no history of injection of horse serum.

Although both agglutinins and hemolysins for sheep erythrocytes occur in normal persons, they rarely reach a titer higher than

1:8. However, the range, which is considered diagnostic for infectious mononucleosis, has not been definitely established. Some believe that a titer as low as 1:16 is significant, while others regard a titer of at least 1:128 to be necessary before making a diagnosis. A rising titer is particularly significant. Strauss and Bernstein[28] reported titers in a series of seventy-five patients with infectious mononucleosis in whom the range was from 1:8 to 1:1024, or higher.

According to Weinstein and Fitzhugh,[29] the heterophile agglutination is helpful in differential diagnosis because leukemias, malignant granuloma (Hodgkin's disease), lymphosarcoma, syphilis, tuberculosis, and agranulocytosis do not produce an increased titer.

Infectious mononucleosis is likely to cause transient, positive, complement-fixation and flocculation tests. Kolmer and co-workers[30] suggest that these reactions occur because the causative agent produces a reagin similar to that produced by Treponema pallidum, Mycobacterium leprae, and Plasmodium vivax.

TYPHUS FEVER

The causative agent of typhus fever is Rickettsia prowazeki, transmitted by lice. Theoretically, it should be possible to demonstrate Rickettsia prowazeki bodies in lice fed on typhus patients, but practically this procedure is too lengthy. The Weil-Felix reaction is the only test of importance in this disease. In 1916, Weil and Felix,[31] working in Eastern Galicia, cultivated a proteus-like organism from the urine of a patient with typhus. This organism was agglutinated by the patient's own serum and by sera from other patients. The strains of organisms isolated from various patients were called "Proteus-X" strains, and the most highly agglutinable of these was Proteus X_{19}. This strain was agglutinated in titers ranging from 1:50 to 1:50,000 by sera from typhus patients and never over 1:25 by sera from nontyphus patients.

Agglutinins for Proteus X_{19} occur about the fourth day of the disease and increase up to the beginning of the third week. They fall rapidly during convalescence and by the fifth month they disappear.

It is important to remember that it is only the agglutination of the O antigen which is diagnostic of typhus fever. Normal agglutinins to the H antigen occur commonly and have no significance.

It is important, therefore, to use organisms from a nonmotile, Proteus X_{19} strain to avoid the results of flagellar agglutinins. Thus, agglutination of a nonmotile strain, which represents only the O antigen, is designated as "Proteus OX_{19}."

ROCKY MOUNTAIN SPOTTED FEVER

This disease is caused by Rickettsia rickettsi, which is transmitted by ticks, Dermacentor andersoni (Western type), and Dermacentor variabilis (Eastern type).

The Weil-Felix reaction is also helpful in diagnosing this disease. It was found by Davis and associates[32] that 77 per cent of sera taken between the tenth and thirty-second days of the disease agglutinated Proteus OX_{19} in a titer of 1:160, or higher. When two samples were taken at suitable intervals, 95 per cent proved positive.

In addition to these two diseases, Mexican typhus, Brill's disease, Toulon typhus, endemic typhus of Manchuria, Moscow, and Australia, and ship typhus of Malaya cause agglutination of Proteus OX_{19}. Tsutsugamushi fever, scrub typhus of Malaya, and mite fever of the East Indies agglutinate Proteus OXK. Slight agglutination of Proteus OX_{19}, OX_2, and OXK is caused by São Paulo fever, boutonneuse fever (exanthematous fever of Marseille), febbre erutiva, tick-borne typhus of South Africa, India, and Kenya, endemic typhus of South Africa, and trench fever.

COLD AGGLUTININS IN VIRUS PNEUMONIA

Autohemagglutination has been considered to be a rare occurrence, associated only with certain blood dyscrasias and liver disease. In recent years, a few authors[33 to 36] have noted the presence of cold hemagglutinins in primary atypical pneumonia. Turner[35] reported from England a series of 22 cases of atypical pneumonia and 24 cases of other infectious diseases. Titers of 1:16, or more, were found in approximately 50 per cent of the cases, whereas none of the other diseases showed a titer greater than 16. Turner and coworkers,[36] in a later series found titers of 1:32 in 50 per cent of 83 cases of atypical pneumonia and titers of 1:128, or higher, in 28 per cent. Of 136 cases of other infectious diseases, the titer was over 1:16 in only four cases, and in no instance was it more than 1:64. The

Commission on Acute Respiratory Diseases[37] of the Army of the United States presented a study of cold agglutinins in 93 cases of atypical pneumonia and 121 cases of other types of respiratory diseases. The data in this article indicates that a titer of 1:64, or more, in a patient ill with a respiratory infection is strong evidence in favor of atypical pneumonia. The titers are frequently low, 1:8 or less, in the first few days of the disease; but if repeated tests reveal rising titers, it is considered significant.

We have noted recently the presence of cold agglutinins in two patients with diseases other than virus pneumonia who had transfusion reactions with homologous blood donated by different donors. Whether or not the cold agglutinins constituted the entire cause of the reactions is unknown; but in one of these cases, subsequent transfusions of homologous blood warmed to 98.6° F. (37° C.) and given slowly did not cause a reaction.

Technic of Determining Cold Agglutinins:

The technic used by the Commission on Acute Respiratory Diseases[37] was as follows:

> All of the sera from each patient were tested on the same day. Human, defibrinated, Group-O erythrocytes were obtained daily from the same donor for all tests. The cells were washed three times with approximately equal volumes of buffered physiologic saline and then packed in the horizontal centrifuge for fifteen minutes at 1500 r.p.m. A 0.2 per cent suspension of packed cells in buffered physiologic saline was used in the test.
>
> Two-fold serial dilutions of the sera, beginning with a 1 to 4 dilution, were made in buffered physiologic saline in 0.5 ml. volumes. To each of these was added an equal volume of 0.2 per cent suspension; the final dilution of serum in the first tube was 1 to 8. After thorough shaking, the racks were kept at approximately 39.2° F. (4° C.) overnight. On the following morning, the tests were removed from the refrigerator one rack at a time and read immediately before warming could occur. A fluorescent source of light and a black background were used. Readings were graded from 4+, which consisted of a tight disk of cells that did not break up readily on gentle shaking of the tube, to 1+, which was the least amount of definite clumping of cells visible to the unaided eye. The end-point was taken as the highest dilution in which definite agglutination occurred

Positive tests were reread after thirty minutes, at room temperature, to eliminate heteroagglutinins or other antibodies as the cause of agglutination. All titers mentioned are final dilutions of serum and are expressed as the reciprocal of that dilution.

Positive sera of previously determined titer were included in each day's tests as a check on the sensitivity of the tests from day to day. In no instance, did these control sera vary more than ±1 dilution on repeated tests.

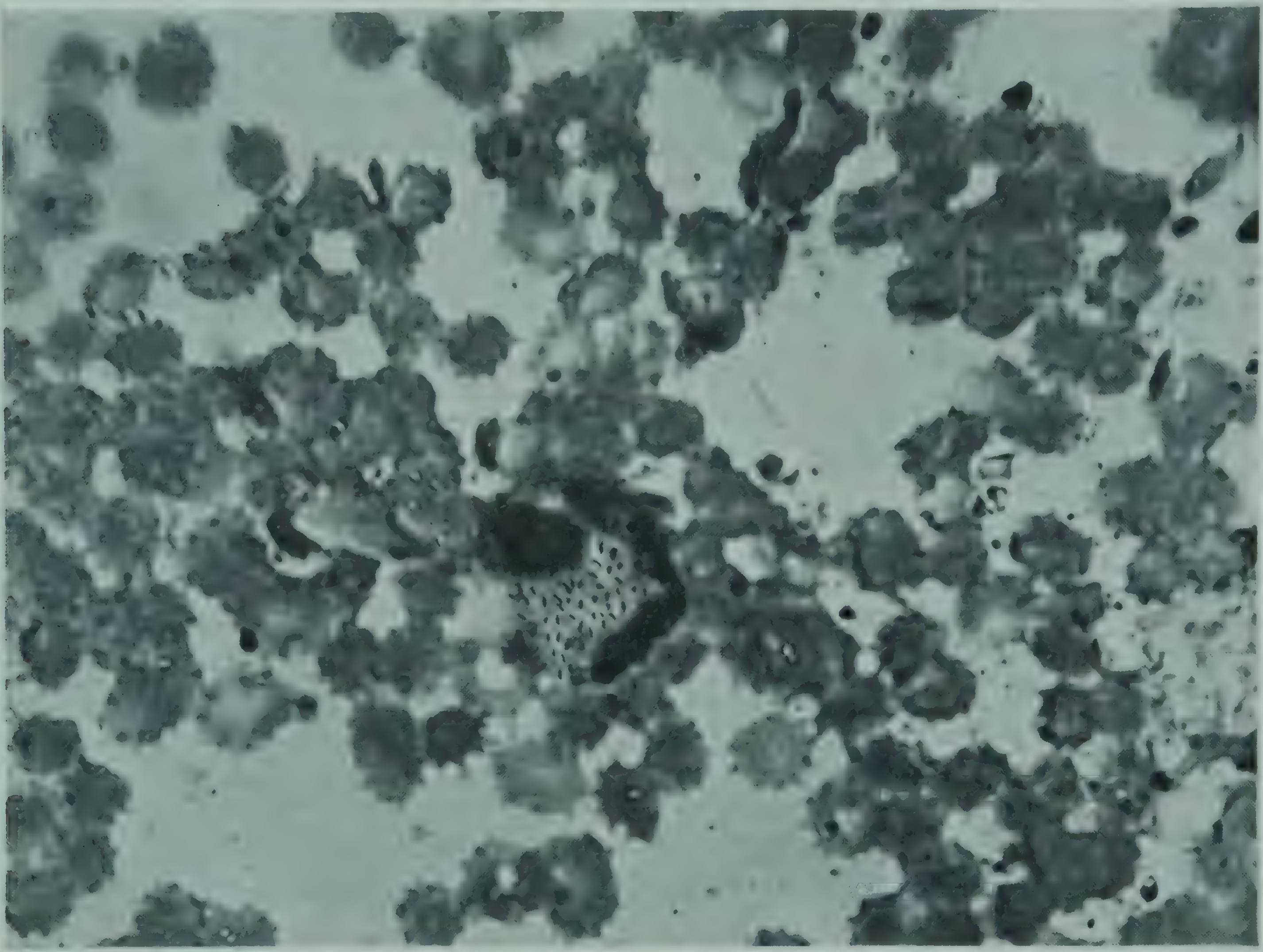

Fig. 5: Brucella-abortus organisms phagocytized by polymorphonuclear leukocyte in determining opsonic index.

OPSONOCYTOPHAGIC INDEX

Determination of the opsonocytophagic index is based upon the ability of a patient's polymorphonuclear leukocytes to phagocytize bacteria. Because of the presence of a disease, past or present, the leukocytes are sensitized to phagocytize the causative organisms. The sensitizing agent is called "opsonin."

This test has been used particularly in brucellosis and tularemia. The test is carried out with the patient's citrated blood and, preferably, killed organisms. The number of bacteria found in each of twenty-five consecutive leukocytes is recorded as follows:

Reaction	*Number of bacteria*
None	0
Slight	1 to 20
Moderate	21 to 40
Marked	41 and above

The following interpretations are used at present:

1. Opsonic index negative, or slightly positive, that is 0 to 20, with negative skin test and negative agglutination reaction: indicative of infection, past or present
2. Opsonic index strongly positive, that is above 40, with positive skin test and with or without positive agglutination: indicative of immunity

Brucellosis:

According to Menefee and Poston,[21] in a series of normal persons with positive skin reactions indicative of previous brucellar infections, about 76 per cent had negative agglutination tests, but about 98 per cent had slight to marked opsonic indices. This suggests that positive opsonic and skin tests are more indicative of previous, minor, brucellar infections than is the agglutination reaction. The opsonocytophagic index is of little, or no, value in acute brucellosis; in fact, brucella organisms have been cultured from acute cases with negative skin test, agglutination, and opsonocytophagic index.

As a diagnostic measure, the opsonic index must be evaluated in connection with agglutination and skin tests. A positive skin reaction, a positive agglutination, and a negative or weakly positive opsonic index, when present together, usually indicate the presence of brucellosis. The degree of phagocytosis (opsonic index) is a relative, but not infallible, measure of the patient's resistance. Huddleson and his co-workers[38] stated that a person with a positive skin test is classified as "infected" if less than 40 per cent of the cells show marked phagocytosis, as "questionably infected" or "immune" if 40 to 50 per cent of the cells show marked phagocytosis, and as "immune" if 60 per cent or more of the cells show marked phagocytosis. This immunity, however, may be short-lived, for the patient may have a different opsonic index a week or a month later.

Tularemia:

This test is carried out in the same manner as for brucellosis, using a suspension of formalin-killed Pasteurella tularensis. In tularemia, the opsonic index must be evaluated in connection with the skin test, agglutination test, and clinical symptoms. In cases where there is crossagglutination between Pasteurella tularensis and Brucella abortus, it may be of help in establishing the diagnosis, provided it is carried out with both organisms.

COMPLEMENT FIXATION

The phenomenon of complement fixation was discovered by Bordet and Gengou in 1901. In 1906, Wassermann introduced his test for syphilis based upon complement fixation.

Complement-Fixation Test

The complement-fixation test is really nonspecific because it is not an antigen-antibody reaction. The antigen is not prepared from Treponema pallidum in the case of syphilis, but is prepared from nonsyphilitic tissues, such as beef heart. The principle of the test rests on the fact that syphilis produces a thermostabile antibody (reagin), which is apparently different from spirochetal antibodies. The reagin in the serum, in the presence of the corresponding antigen (a beef-heart extract), will combine with the complement, a thermolabile substance found in normal blood, and remove it from solution. This is called "fixation of complement." The fixation of complement is then determined by a "hemolytic system," so-called because it uses specially prepared sensitized erythrocytes (sheep cells) to demonstrate the reaction. If the complement has been fixed by the antibody (reagin) in the presence of antigen, no hemolysis occurs, which indicates a positive reaction. If no reagin is present, the complement is not fixed, and the cells are hemolyzed. This is a negative reaction because reagin cannot be demonstrated.

The complement-fixation test has been used for years in the diagnosis of syphilis and certain other diseases, but for syphilis it has now largely been replaced by the simpler flocculation tests.

In Various Diseases:

Syphilis: As indicated above, the Wassermann complement-fixation test is in no way specific in the bacteriological sense; its basis is essentially empirical. It has been found in practice, however, to be one of the most useful and reliable methods in the laboratory diagnosis of syphilis. Its diagnostic significance depends upon the care with which the test is done and the correlation with clinical findings. Unfortunately, the complement-fixation test for syphilis gives a certain percentage of false-positive results in leprosy and malaria.

The figures from a report of the United States Public Health Service, prepared by Cummings and group,[39] indicate the specificity of the Wassermann reaction in various stages of syphilis (Table 8). Modifications of the Wassermann reaction of various workers using the test are indicated by numbers.

TABLE 8: PERCENTAGE OF SERA REACTING POSITIVELY

	Untreated Primary Syphilis	*Untreated Secondary Syphilis*	*Varying Treatment of Late Syphilis*
1.	65.9	100	66.6
2.	65.9	100	72.1
3.	82.5	100	86.4
4.	69.8	100	58.0

Yaws and Pinta: Yaws, also known as "framboesia tropica," is caused by Treponema pertenue. It is not certain whether or not yaws is a tropical form of syphilis; in any event, the causative agent is undistinguishable from Treponema pallidum. Likewise, the status of pinta as a separate disease entity is not fully decided. Some believe it is a late stage of yaws and call it "vitiliginous yaws." Positive Wassermann and flocculation reactions are found in both diseases. Allen and Goodale[40] reported 76 per cent of positive Kahn tests in twenty-nine cases of pinta seen on Guam.

Gonorrhea: The use of the complement-fixation test in gonorrhea requires extreme care and a sensitive polyvalent antigen. Since the test is often negative in anterior urethritis, it is not used for the diagnosis of this condition. Other points that must be remembered are the following: 1 The reaction does not become positive for at least two weeks after the infection starts; 2 it is generally positive in those cases in which there are complications, such as gonorrheal arthritis; 3 it remains positive for about three months after clinical cure; 4 a single negative reaction is of little significance; 5 two or more negative reactions over a period of a month are of significance in ruling out the disease; 6 a positive reaction that becomes negative about three months after clinical cure is strong evidence in favor of a real cure; 7 a positive reaction indicates recent activity of the gonococcus; and 8 gonococcus vaccine may cause a false-positive test.

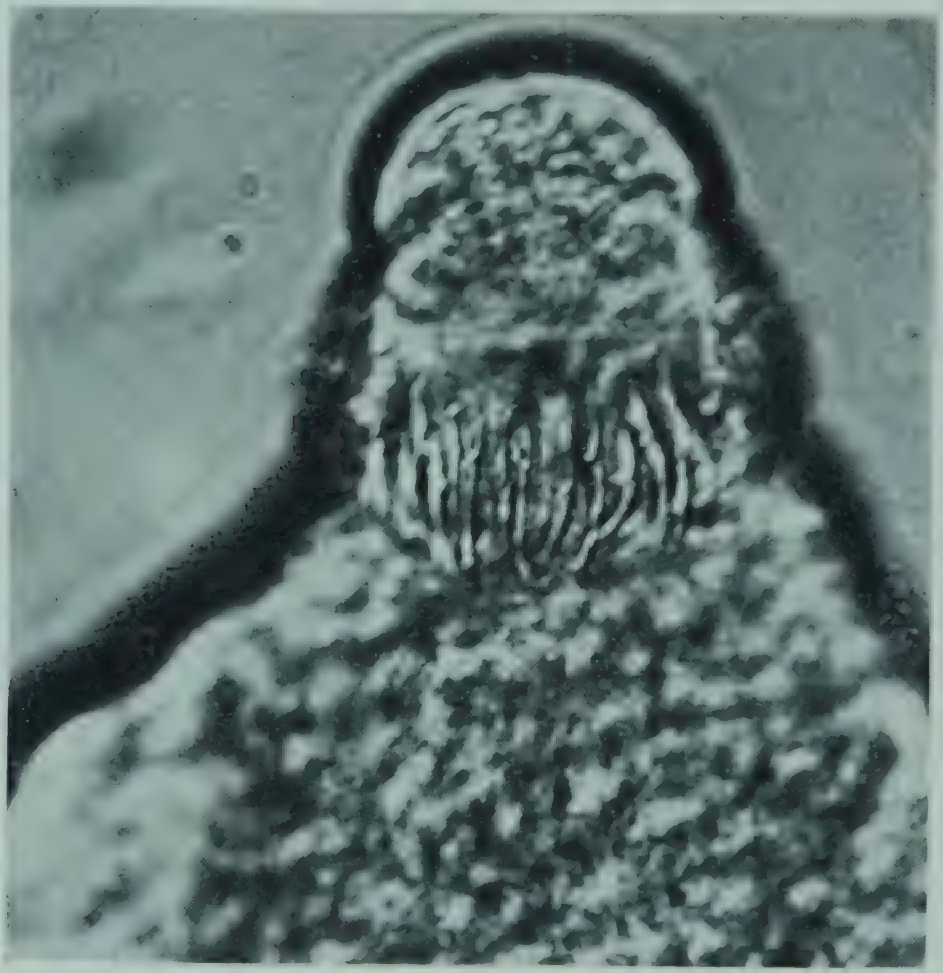

Fig. 6: Scolex of hydatid cyst with head everted.

The complement-fixation test for gonorrhea is most helpful in distinguishing complications in the male genital system (epididymitis, prostatitis, and vesiculitis) and in arthritis.

Hydatid Cyst: The complement-fixation test is positive in from 80 to 90 per cent of the cases when fresh antigen is used. The best antigen is uncontaminated cyst fluid. Since complement-fixation reactions, precipitin tests, and skin tests in this disease apparently are positive in the presence of any of the taenial parasites, the occurrence of false-positive results is unavoidable. Consequently, when the test is used, it must be carefully interpreted.

A positive test, which becomes negative following the removal of a cyst, is significant and indicates that no more cysts are present.

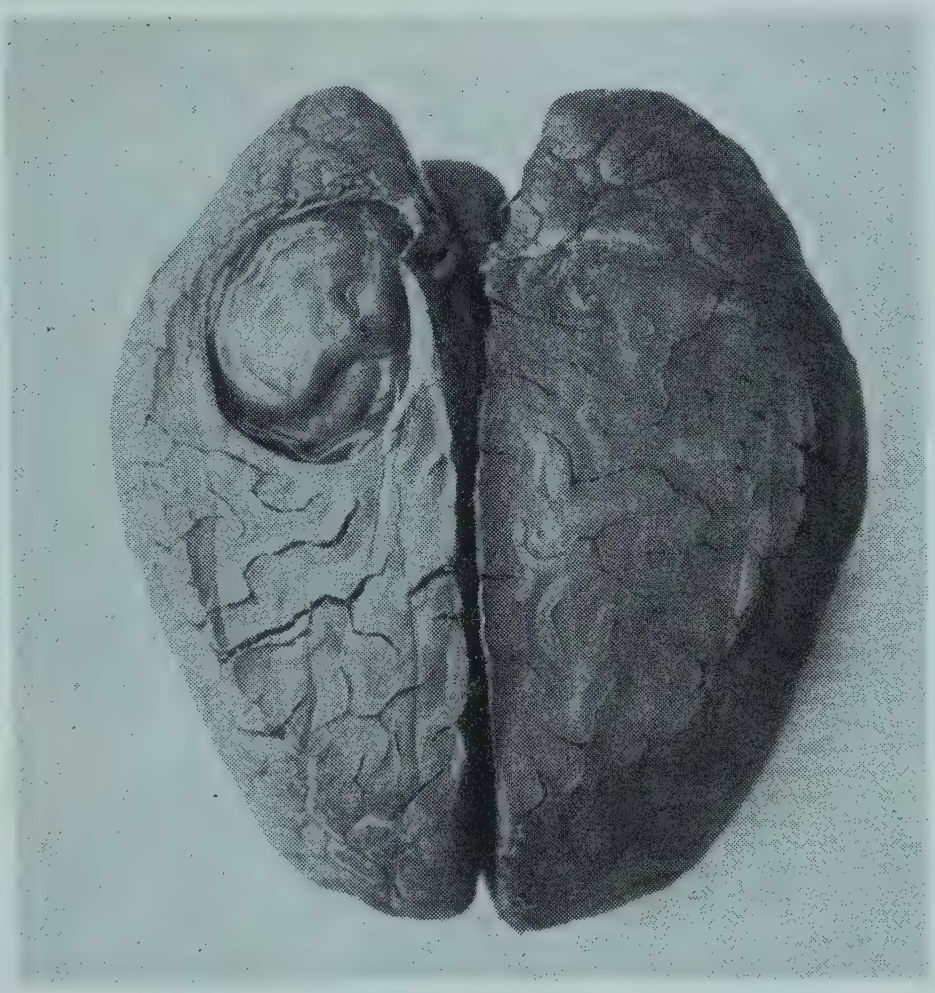

Fig. 7: Hydatid cyst of brain.

Amebiasis: Craig[41] has reported a satisfactory complement-fixation test for this disease by using an alcoholic extract of cultures as the antigen. He claims positive reactions as high as 90 per cent in all types of amebiasis. Craig also is of the opinion that the test is of value following treatment with amebacides. Negative reactions over a period of weeks indicate a cure. In active amebic dysentery, it is easier and quicker to examine the fresh stool, but only the reports of experienced workers should be accepted.

Lymphopathia Venereum: Complement-fixation tests were not considered satisfactory until egg-yolk cultures of the virus were used as antigen. Rake and co-workers,[42] in using this antigen (lygranum), found that the test became positive within a week after the onset of disease and yielded almost 100 per cent positive results. A small number of false-positive reports is noted, some of which may be clinically unrecognized cases of the disease. According to Rake, Eaton, and Shaffer,[43] the viruses of psittacosis and meningopneumonitis share a common antigenic factor with that of lymphopathia venereum. This results in cross-complement-fixation reactions and may account for some false-positive results.

Virus Diseases: (See p. 280.)

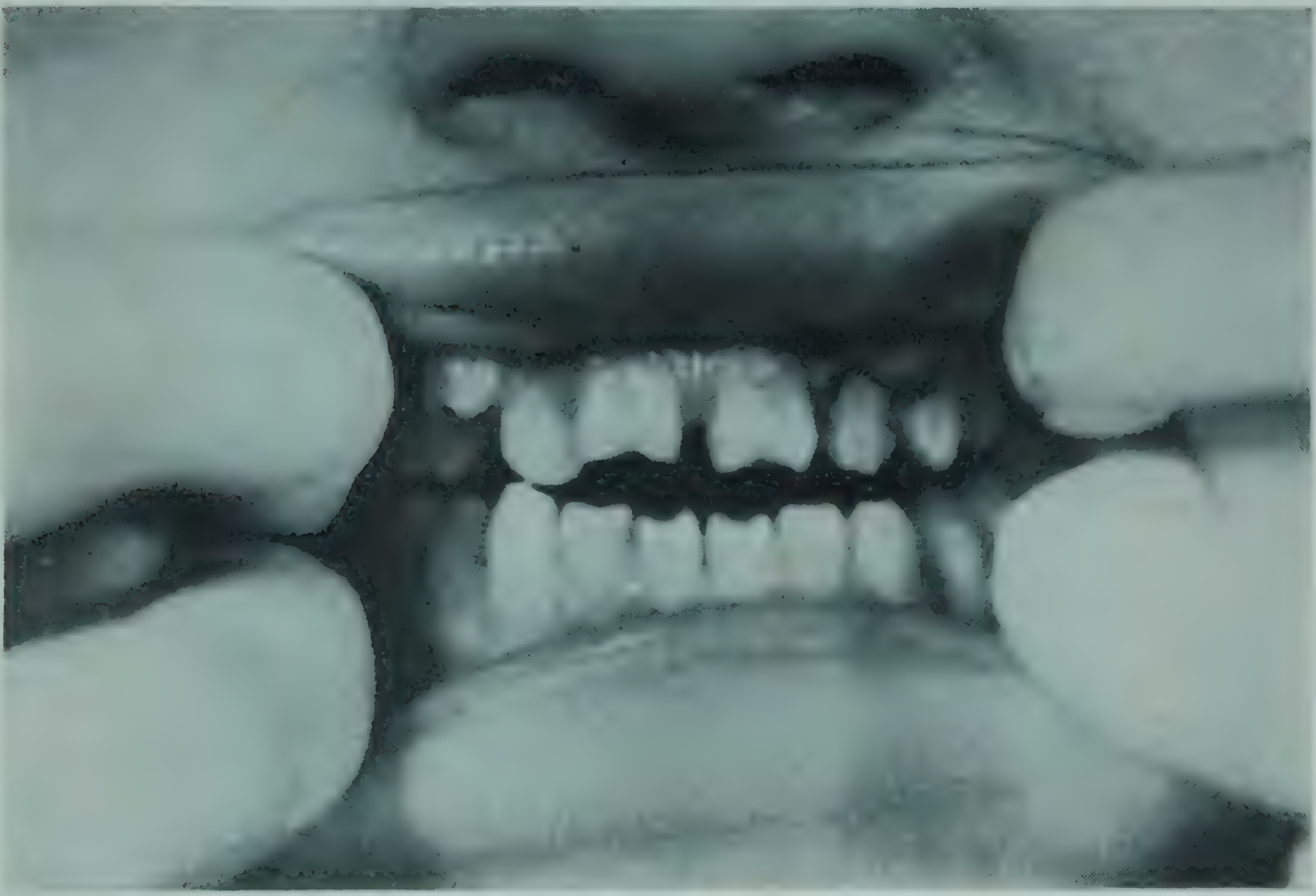

Fig. 8: Hutchinson's teeth in patient with congenital syphilis.

FLOCCULATION REACTIONS

The flocculation reaction, as stated before, has largely replaced the complement-fixation test in the diagnosis of syphilis. It has certain obvious advantages for routine diagnostic purposes because it involves the use of only two reagents, serum and antigen. Further, it is more easily standardized, more easily performed, and the results appear to be equally as good. The flocculation test usually becomes positive two to three weeks after the primary infection.

A number of flocculation tests have been introduced. Among the authors of these tests are Michaelis, Meinnecke, Sachs and Georgi, Dreyer and Ward, Kahn, Kline, Eagle, Hinton, Boerner and Lukens, and Laughlen and Mazzini. A report from the United States Public Health Service, submitted by Cummings,[39] gives comparative figures of the results of different flocculation tests (Table 9).

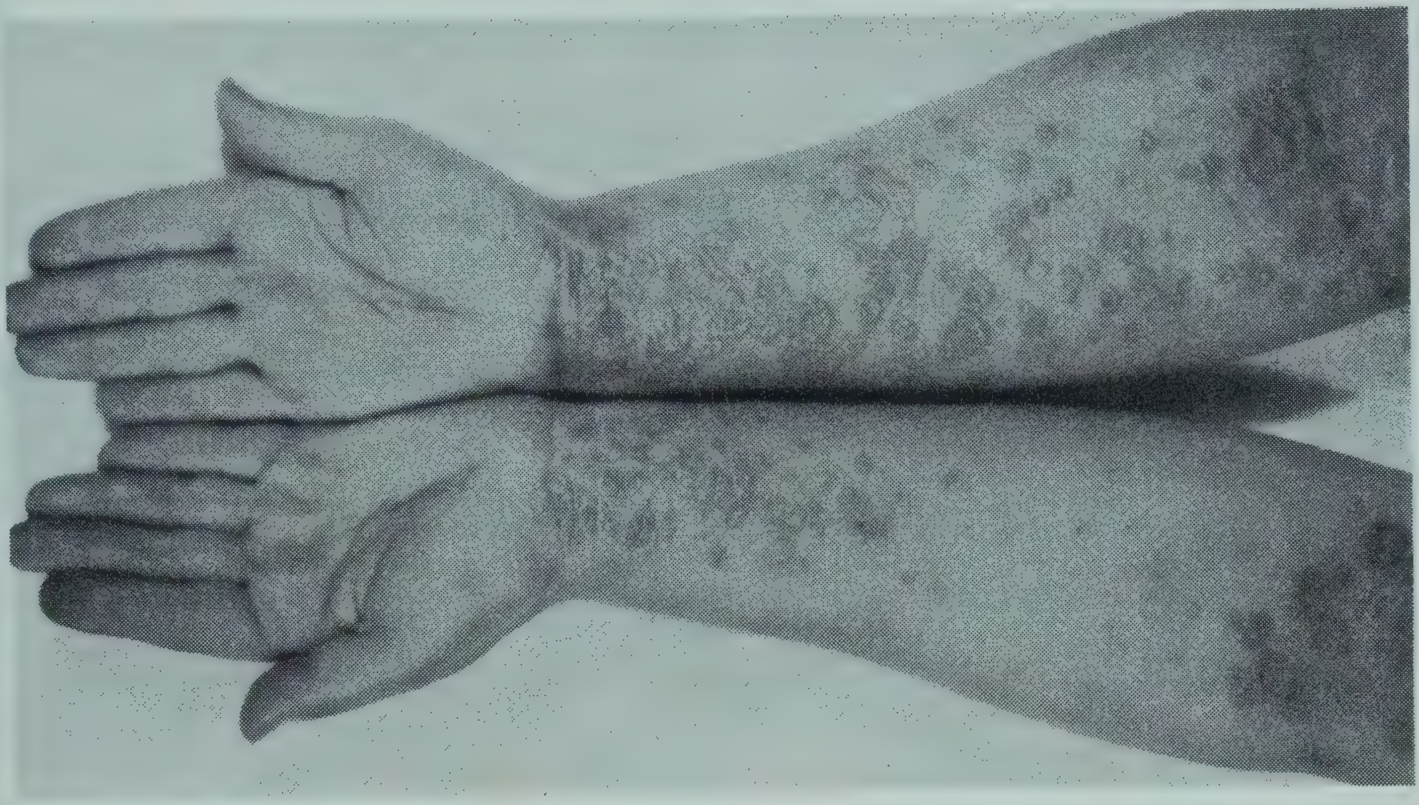

Fig. 9: Secondary syphilis.

TABLE 9: COMPARISON OF FLOCCULATION TESTS

Percentage of Sera Reacting Positively

Tests	*Untreated Primary Syphilis*	*Untreated Secondary Syphilis*	*Varying Treatment of Late Syphilis*	*Normal Non syphilitic*
Kahn tests:				
1.	76.0	100.0	76.9	0.0
2.	82.9	100.0	84.3	3.3
3.	80.5	100.0	83.0	0.7
Other flocculation tests:				
1.	72.1	100.0	82.4	2.0
2.	81.0	100.0	84.5	0.7
3.	58.1	98.4	64.5	3.3
4.	74.4	100.0	71.5	0.0
5.	72.1	98.5	83.6	1.3
6.	70.7	98.4	63.1	0.7

The Committee on the Evaluation of Serodiagnostic Tests[44] under the United States Public Health Service gave the following figures for the sensitivity of certain flocculation tests using sera from syphilitic patients of various stages both treated and untreated: Boerner-Lukens, from 62.9 to 68.3 per cent; Eagle, 72.1 per cent; Kahn standard, 62.2 per cent; Kahn presumptive, 74.6 per cent; Kline diagnostic, 62.6 per cent; Laughlen, 69.5 per cent; and Mazzini, 78.6 per cent.

Reports of Flocculation Tests:

A clinician should become familiar with the method of reporting serological tests in his area. The use of + + + +, + + +, + +, +, and ± to indicate the strength of the reaction is used in some places; other places use such expressions as "strongly positive," "weakly positive," "doubtful," and "negative." Some such method of giving the clinician an idea of the strength of the reaction is advisable. If a quantitative test is used, the dilution factor should be clearly expressed by the serologist.

False-Positive Reactions:

False-positive reactions may occur as a result of (1) technical errors, (2) inherent abnormalities of the serum, and (3) certain diseases other than syphilis.

Technical Errors: Technical errors may happen in any laboratory, but the number of false-positive tests should be kept to a minimum. In proper hands, large series of tests may be done without a false positive. Since the flocculation tests are more sensitive than the complement-fixation tests, false-positive tests are more likely to occur with the former test.

Inherent Abnormalities of Serum: A few false-positive results are reported regardless of the care and technic used in carrying out the test. This problem has been investigated by Cardon and coworkers,[45] who believe that hyperproteinemia and hyperglobulinemia may be the cause of false-positive reactions in both complement-fixation and flocculation tests, and suggest that the blood proteins be examined in such cases. Complement-fixation tests are less likely to give false-positive tests under these conditions.

Certain Diseases Other Than Syphilis: False-positive reactions occur in certain diseases other than syphilis, and they may occur with either complement-fixation or flocculation tests. The most important of these diseases are as follows:

Leprosy
Malaria
Infectious mononucleosis
Rat-bite fever
Relapsing fever
Leptospirosis
Treponematoses
Vaccination (smallpox)

Other conditions that yield occasional false-positive tests are upper respiratory infection, lupus erythematosus, atypical pneumonia, tuberculosis, and febrile conditions.

It is extremely unfortunate that false-positive, complement-fixation and flocculation tests occur in other diseases than syphilis. It behooves the clinician to take a thorough history, do a complete physical, and employ laboratory and x-ray aids to diagnose any other condition that may be present. It is, of course, possible to have syphilis present in addition to another disease. This makes the problem particularly difficult if there is no evidence of syphilis in the history or physical examination.

False-Negative Reactions:

During the primary stage of syphilis, the serological test is not always positive. Later, it usually becomes positive, particularly in the secondary stage. Late syphilis and tabes dorsalis sometimes yield false-negative tests as high as 20 per cent. Likewise, up to 40 per cent of negative tests may occur in newborn, congenital, syphilitic infants with evident lesions of syphilis. Consequently, when the serological test does not confirm the physical findings, the clinician must assume the responsibility for appropriate treatment.

PRECIPITIN TESTS

Trichinosis appears to yield the best results with the precipitin test, using Trichinella spiralis as an antigen. The test becomes positive between the second and fourth weeks and is positive in as high as 90 per cent of the cases. It is important that the serum be sterile because bacterial growth confuses the result. The skin test should be done in conjunction with the precipitin test.

The precipitin test has been used in diagnosing meningococcus meningitis by adding antimeningococcal serum to cerebrospinal fluid. Precipitins occur with hydatid cysts and in various other parasitic disease, but the test has not proved of practical value. It is of real help in identifying blood stains in medicolegal investigations.

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CHAPTER 4

Urine

PHYSIOLOGY OF URINE FORMATION

In the formation of urine, it is believed that two processes take place, namely simple filtration and selective absorption. Nonselective filtration, which is a simple physical process, takes place through the glomerular capillary endothelium and the squamous epithelium which envelops the glomeruli. Selective reabsorption by the tubules concentrates the urine; and certain products, including glucose and some water, are transferred back into the bloodstream.

NORMAL URINE

There is a wide range in the physical and chemical composition of normal urine because of the variation in fluid and food intake, physical activity, and body temperature. The important normal constituents, together with the significance of changes, will be discussed under the appropriate headings. Table 1 shows the constituents of normal urine.

VOLUME

The normal amount of urine secreted in twenty-four hours by an adult on normal fluid intake is from 1000 to 1500 cc. Children

TABLE 1: COMPOSITION OF TYPICAL NORMAL URINE (DAILY EXCRETION) *

Constituent	*Amount (Gm.)*
Water	1200.00
Solids	60.00
Urea	30.00
Uric acid	0.70
Hippuric acid	0.70
Creatinine	1.20
Indican	0.01
Oxalic acid	0.02
Allantoin	0.04
Amino-acid nitrogen	0.20
Purine bases	0.01
Phenols	0.20
Chloride as sodium chloride	12.00
Sodium	4.00
Potassium	2.00
Calcium	0.20
Magnesium	0.15
Sulfur, total, as sulfur	1.00
Inorganic sulfates as sulfur	0.80
Neutral sulfur as sulfur	0.12
Conjugated sulfates as sulfur	0.08
Phosphate as phosphorus	1.10
Ammonia	0.70

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* Hawk, P. B., Oser, B. L., and Summerson, W. K.: Practical Physiological Chemistry. The Blakiston Company, Philadelphia, 1943, p. 721.

secrete three to four times as much as adults per kilogram of body weight. The output of urine is increased with increased fluid intake and with a protein-high diet. The secretion of urine during the daytime is more than twice that at night, even though the fluid intake is the same for each period. It is well known that sweating as a result of high temperature or exercise reduces the output of urine. The loss of fluid by diarrhea or vomiting also decreases urinary excretion.

Nocturia:

The secretion of more than 500 cc. of urine at night with a specific gravity of less than 1.018 is called "nocturia." Increased frequency of urination at night is not nocturia unless the total volume is more than 500 cc. and the specific gravity is less than 1.018. Nocturia occurs characteristically in nephritis.

Polyuria:

The increased secretion of urine is known as "polyuria." Polyuria may occur under physiological conditions, such as the following: after excessive fluid intake; after drinking coffee, tea, whisky, or beer; after taking diuretics; after intravenous saline and glucose solutions; and during excitement, as in a football player before an important game.

Polyuria is seen in certain diseases, such as diabetes insipidus, diabetes mellitus, and certain stages of chronic nephritis. It is seen to a lesser extent in myxedema, acromegaly, tabes dorsalis, paralysis agitans, and tumors of the brain and spinal cord.

Oliguria:

Decreased excretion of urine is known as "oliguria." Oliguria is seen in certain physiological conditions, such as in decreased fluid intake, excessive sweating, and increased salt intake. Under pathological conditions, it occurs following diarrhea and vomiting, and obstruction of the ureters by pressure from without or calculi within. It is also seen in acute nephritis and nephrosis, cardiac deficiency, acute febrile diseases, portal cirrhosis, acute yellow atrophy of the liver, peritonitis, acute intestinal obstruction, and poisoning by agents that damage the kidneys.

Anuria:

Complete suppression of urinary excretion by the kidneys is called "anuria." Anuria is found as a result of any disease that causes extensive damage of both kidneys, or when function is completely abolished, as in poisoning with bichloride of mercury.

Under certain conditions, the passage of urine is obstructed by pathological processes in the lower urinary tract, even though the kidney is able to secrete the urine. This is known as "symptomatic anuria"; it occurs as a result of obstruction of both ureters or the urethra.

COLOR

The urine is normally yellow or amber owing to urochrome, a urinary pigment, the source of which is not known. In addition, small amounts of uroerythrin, urobilin, and hematoporphyrin are found. The depth of the color depends upon the concentration of the urine. With a large fluid intake, the urine is pale; with a low fluid intake the urine is highly colored (dark amber). The color of the urine is influenced further by certain pathological processes and by the administration of drugs.

Color changes due to pathological processes are summarized in Table 2.

TABLE 2: COLOR CHANGES OF URINE DUE TO PATHOLOGICAL PROCESSES

Color	*Cause*
Orange-yellow to green-brown	Bilirubin from obstructive jaundice
Clear red to red-brown	Hemoglobinuria in paroxysmal hemoglobinuria, black-water fever, incompatible blood transfusion, severe burns, exposure to cold, or intra-abdominal hemorrhage.
Smoky red to brown	Unhemolyzed red cells from hemorrhage in urinary tract
Port-wine color	Hemochromatosis, hematoporphyrinuria, or hemolytic jaundice
Brown with black tinge	Melanin from malignant melanoma or alkaptonuria
Dark brown	Urobilin in liver infection, malaria, pernicious anemia, or acute infections

Color changes due to drugs and chemicals, are summarized in Table 3.

TABLE 3: COLOR CHANGES OF URINE DUE TO DRUGS AND CHEMICALS

Color	*Cause*
Green-blue	Methylene blue or thymol
Dark brown to black	Carbolic acid, guaiacol, creosote, salol, or resorcin poisoning
Deep red	Pyramidon, prontylin, or rose bengol
Pink (alkaline urine)	Administration of phenolsulphonphthalein in kidney-function test
Orange-red	Pyridium
Deep yellow	Santonin, senna or chrysarobin
Yellow-green	Acriflavine

Turbidity:

Turbidity may be caused by the presence of pus cells (pyuria), and in an alkaline urine by phosphates and carbonates. A pink turbidity, which settles out, is due to salts of uric acid. A faint turbidity is sometimes noted in the presence of large numbers of bacteria. A milky urine (chyluria) is caused by the admixture of lymph as in filariasis, and in rupture of lymph vessels into the urinary tract. An opalescent urine may be seen in lipuria, which is seen after ingestion of food containing a great deal of fat, after crushing injuries that damage fat tissues, and after alcohol and phosphorus poisoning.

ODOR

Volatile acids are responsible for the normal aromatic odor of fresh urine. After standing, it develops an ammoniacal odor, which is said to be due to the breakdown of urea. Infected urine may have a foul odor, owing to the presence of bacteria and pus cells. Characteristic odors are imparted by menthol, which causes a peppermint odor; asparagus, which imparts a disagreeable, somewhat acrid odor; and sandalwood oil, tolu, and saffron, which impart peculiar spice-like aromas.

SPECIFIC GRAVITY

Urine is largely a solution of urea and certain minerals in water; consequently, the specific gravity must be greater than 1.000. The specific gravity of the urine is dependent upon the concentrating ability of the kidneys. The normal range is from 1.010 to 1.030. The morning specimen shows the greatest concentration, and the specific gravity is usually 1.020, or higher. It may be lower if the fluid intake is large or if the salt intake is low, and higher if there has been water loss by sweating, vomiting, or diarrhea.

Specific gravity is high in diabetes mellitus, acute glomerulonephritis, fever, sweating, vomiting, and diarrhea. It is low in diabetes insipidus and chronic nephritis. In chronic nephritis, the tubules are damaged and unable to reabsorb water, and thus to concentrate the urine. This is the basis for such kidney function tests as the concentration and dilution tests.

REACTION

The reaction of normal fresh urine is usually acid, owing to acid sodium phosphate and free organic acids, which give it a pH of about 6.0. Urine becomes alkaline on standing, owing to the formation of ammonia from urea.

The reaction is strongly acid in acidosis, diabetes mellitus, gout, lithiasis, acute articular rheumatism, chronic nephritis, leukemia, and scurvy. It is also acid after ingestion of acids, saccharin, or a protein-high diet, and after taking such medicines as mendelic acid, ammonium chloride, ammonium mandelate, and ammonium nitrate.

The reaction is alkaline after ingesting salts of organic acids and alkaline carbonates, in certain anemias and certain cases of debility, following resorption of alkaline transudates, and after transfusions of blood, frequent vomiting, and a prolonged cold bath.

17-KETOSTEROIDS (URINARY ANDROGENS)

In the following discussion, reference is made to Hawk, Oser, and Summerson.[1] Human urines contain steroids carrying ketonic oxygen at C_{17}; some of these are phenolic, while others are neutral. The latter are called "17-ketosteroids." The principal members of the group are androsterone (III), etiocholanolone (V), esoandrosterone (IV), and dehydroisoandrosterone (VI). The first two are derived in part from the testis, while all four represent excretion products of some of the steroids of the adrenal cortex. Consequently, the measurement of the 17-ketosteroid output provides an index of testicular and adrenocortical activity.

Males between twenty and forty years of age excrete about 15 mg. of 17-ketosteroids in twenty-four hours, while women of the same age group excrete about 10 mg. in twenty-four hours. In children under eight years of age, the excretion is less than 1 mg. per twenty-four hours, and from this age on there is a gradual increase to adult values. A corresponding decrease is noted in old age. Orchidectomy results in a decrease of 17-ketosteroids from 15 to 10 mg. in twenty-four hours, but oophorectomy does not affect the output in the female. It would seem, therefore, that about 5 mg. come from the testis and 10 mg. from the adrenal cortex. The human ovary is not a source of 17-ketosteroids.

In eunuchoidism, the values are decreased and may be as low as the surgical castrate (10 mg. in twenty-four hours). On the other hand, in masculinizing tumors of the interstitial cells of the testis, the output may reach as high as 800 mg. in twenty-four hours. In Addison's disease in the male, the urinary output drops to from 1.2 to 6.4 mg. in twenty-four hours. This represents the amount put out by the testis. In the female with the same disease, there is practically no output of 17-ketosteroids, since the adrenal cortex constitutes the only source. In pituitary basophilism not associated with carcinoma of the adrenal cortex, normal or only slightly elevated, 17-ketosteroid values are obtained. When carcinoma of the adrenal cortex is present, a much higher excretion is noted (40 to 288 mg. in twenty-four hours). In the adrenogenital syndrome, simple hyperplasia of the adrenal cortex results only in a moderately high 17-ketosteroid output, up to 100 mg. in twenty-four hours. In panhypopituitarism with a general decreased excretion of all anterior-lobe hormones, the production of 17-ketosteroids is low (0 to 3 mg.).

PROTEINS

Albumin and Globulins:

Albumin and the globulins (pseudoglobulin and euglobulin) are the most important protein constituents of the urine. Definitively, the term "proteinuria" should be used, but precedent and tradition will probably keep the more familiar term "albuminuria" in medical literature. Although a small amount of the above-mentioned proteins (75 mg. in twenty-four hours) is present in normal urine, it is not detected by ordinary means. Presumably, the source of urinary albumin and globulins is the blood plasma.

Physiological Albuminuria:

Albumin is found in the urine as a result of protein-high intake, particularly egg protein, and in the presence of hemoglobinuria and methemoglobinuria. Albumin, in these instances, passes through normal kidneys, and the condition can therefore be called "physiological albuminuria."

Orthostatic albuminuria, which occurs with activity in the upright position and disappears on lying down, was formerly thought to be strictly functional. It is now known that nephritis may begin in such kidneys; hence, the increased permeability must be interpreted as indicative of early renal disease.

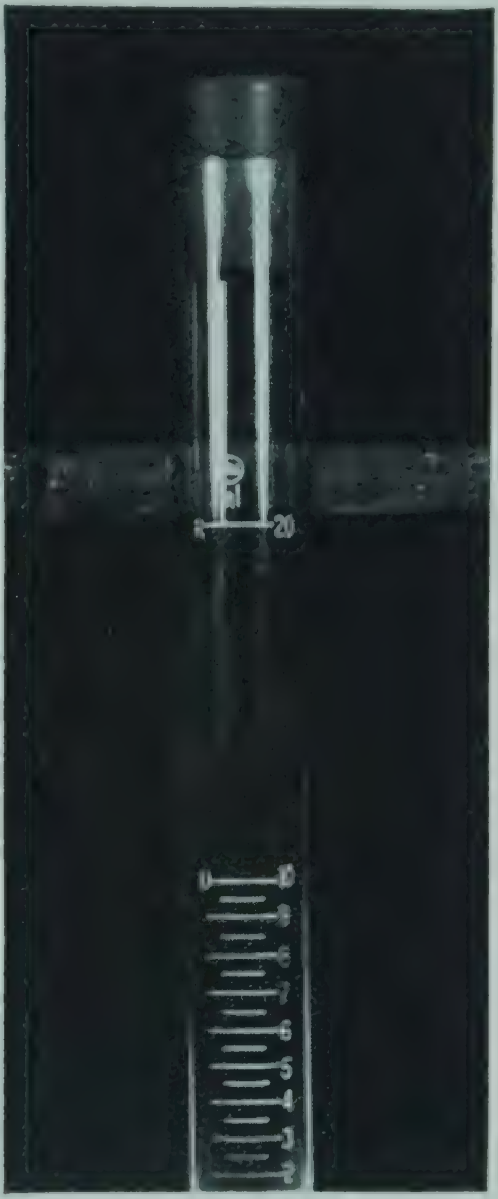

Fig. 1: Esbach tube for quantitative determination of albumin in urine.

Transient albuminuria during pregnancy or following mental strain, exercise, and prolonged exposure to cold is thought to be due to a temporary, increased, glomerular permeability, owing to congestion of the capillaries.

Albuminuria from Renal Disease:

Albuminuria is found in all stages of nephritis, nephrosis, and nephrosclerosis (see pp. 444-454) when glomerular and tubular capillaries are involved. Nephrosclerosis is present in essential hypertension and secondary hypertension. Albuminuria is not always present in benign nephrosclerosis.

Certain infectious processes involving the kidney produce albuminuria, namely tuberculosis, pyelonephritis, and septic emboli. Noninfectious processes, such as polycystic disease, hemorrhage, incompatible transfusions, and amyloidosis, cause albuminuria.

Albuminuria is also the result of systemic disease, which primarily injures the tubules, such as diabetes mellitus, jaundice, fever, hyperthyroidism, and certain blood diseases.

Chemical injury of the kidneys by bichloride of mercury and toxic drugs causes albuminuria.

Albuminuria from Extrarenal Disease:

Infections of the urinary tract also produce a positive test for albumin. These are prostatitis, urethritis, cystitis, ureteritis, and pyelitis. Calculi of the kidney pelves, ureters, and bladder also cause albuminuria.

Method of Reporting Positive Albumin Tests:

Qualitative albumin tests are usually reported in such a way as to give an approximate indication of the amount of albumin. They are most often reported in one of the ways shown in Table 4 (either three or four gradations).

TABLE 4: TERMS USED IN REPORTING ALBUMIN IN URINE

S. P. T. (slightest possible trace)	+	1
S. T. (slight trace)	++	2
T. (trace)	+++	3
H. T. (heavy trace)	++++	4

NONHEMATOGENOUS PROTEINS

Bence-Jones Protein:

This is an endogenous protein, which is normally found in the bone marrow and in the leukocytes. It occurs in the urine and in the blood and exudates of a patient with multiple myeloma in from 60 to 80 per cent of cases, particularly late in the disease. It is occasionally found in the urine of patients with osteogenic sarcoma, osteomalacia, and bone metastases of carcinoma.

Miscellaneous Proteins:

Proteoses have been found in the urine during pneumonia, diphtheria, ulcerative colitis, carcinoma, dermatitis, osteomalacia, atrophy of the kidneys, and absorption of suppurative processes.

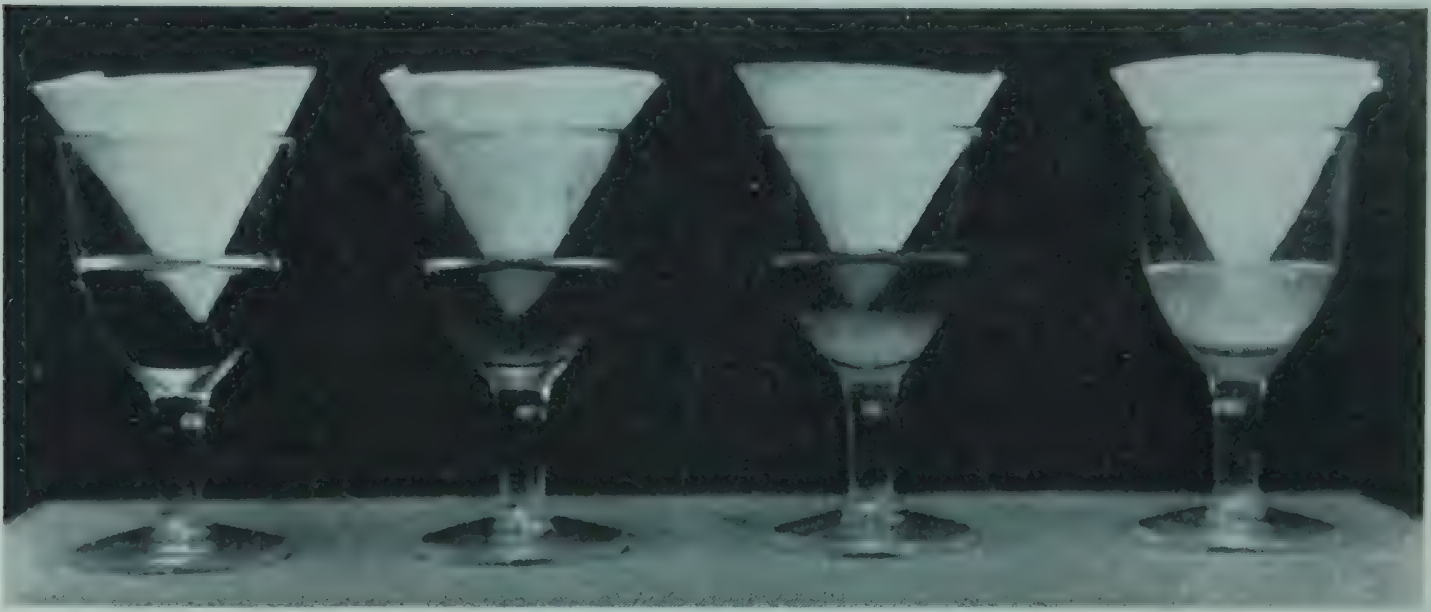

Fig. 2: Test for albumin. From left to right: negative, slight trace, trace, and heavy trace (sulfosalicylic acid technic).

Mucin and mucoid are glycoproteins, which are of little clinical significance, but may be present as a result of urinary-tract infection.

Nucleoprotein occurs in the urine during infections of the urinary tract, particularly pyelonephritis, pyelitis, and cystitis.

NONPROTEIN NITROGENOUS PRODUCTS

Urea:

As stated previously, urea formation appears to take place exclusively in the liver, presumably from ammonia liberated by oxidative deamination of amino acids. From 80 to 90 per cent of the total nitrogen of human urine is in the form of urea. An adult male excretes about 30 gm. in a twenty-four hour period.

A decrease in the excretion of urea in the urine is noted in impaired liver function (hepatitis, acute yellow atrophy, or cirrhosis) acidosis, myxedema, and severe kidney disease. In fact, the ability of the kidneys to excrete urea (urea-clearance test) is the most valuable simple test of renal function.

An increase in excretion of urea is found as a result of increased catabolism, in febrile and wasting diseases, and in absorption of exudates, as in pneumonia or suppurative processes.

Ammonia:

Ammonia, next to urea, is quantitatively the most important end product of protein metabolism. Normally from 2.5 to 4.5 per cent of the total urinary nitrogen is excreted as ammonia. This amounts to about 700 mg. per day.

The kidneys constitute the chief source of ammonia in the urine, as shown by Nash and Benedict.[2] This appears to be proved by the fact that following the extirpation of the kidneys, the blood ammonia does not increase. It is formed apparently by the tubular epithelium, probably from glutamine in the blood. The blood ammonia, on the other hand, appears to come from the tissues, particularly the muscles, and is converted readily to urea. Thus, the blood ammonia remains too low to account for any great part of the urinary ammonia. Urinary ammonia is computed as ammonium salts.

Urinary ammonia is increased in marked diabetic acidosis because it neutralizes diacetic and oxybutyric acids in the kidneys and is excreted. It is also increased in pernicious vomiting of pregnancy, after taking mineral acids, and in cases of liver damage, which reduce the formation of urea.

When the *p*H of the blood is increased, as in alkalosis or after the administration of alkalis, ammonia excretion is reduced.

Uric Acid:

The excretion of uric acid is subject to wide variation, but a normal range is considered to be from 0.5 to 1 gm. in twenty-four hours. Uric acid is apparently formed in the liver, and over half of it is presumably destroyed in the liver. Some is retained in the blood, and the remainder is excreted as urates of ammonium, potassium, and sodium.

Uric-acid excretion is increased in leukemia, polycythemia vera, liver disease, febrile diseases, and eclampsia, and after x-ray therapy and absorption of exudates.

It is decreased in the urine before an attack of gout and is increased during the attack. In gout, the kidney apparently loses its ability temporarily to eliminate uric acid. Salicylates and cinchophen increase the output of uric acid in gout by increasing the activity of the kidneys.

The amount of uric acid in the urine is important in relation to the formation of uric-acid calculi. Alkali carbonates and citrates, which decrease the acidity of the urine, increase its ability to keep uric acid in solution and decrease the chances of calculus formation.

Creatinine:

Creatinine is the anhydride of creatine and is found in the urine in amounts varying from 1 to 1.25 gm. in twenty-four hours. It is a product of endogenous metabolism.

Urinary creatinine is said to be increased in typhoid fever, typhus, tetanus, and debilitating diseases. It is decreased in renal insufficiency, anemia, chlorosis, paralysis, muscular atrophy, and leukemia.

Creatine:

Creatine occurs in very small amounts, or not at all, in the urine of normal adults. It is found in larger amounts in the urine of children and pregnant women. Although it has no great clinical significance, creatine is in the urine in fever, disintegration of muscle tissue, malnutrition, and fasting.

Amino Acids:

The range of amino acids in the urine of adults is from 0.4 to 1.0 gm. per twenty-four hours. Amino acids are absorbed from the small intestine following hydrolysis of protein. Those that are not deaminized in the liver or synthesized into protein in the tissues are excreted in the urine.

Increased excretion in the urine is found in diseases associated with tissue destruction, such as typhoid fever, diabetic acidosis, acute yellow atrophy, eclampsia, and liver damage from poisons.

SUGARS

Traces of sugars, particularly glucose, may occur in normal urine, but they are not detected by ordinary qualitative methods. The occurrence of any sugar in the urine is called "glycosuria"; while the presence of glucose is correctly called "glucosuria," although it is commonly spoken of as "glycosuria." Other sugars that may be present in the urine are pentose, galactose, lactose, and levulose.

Glucose:

When glucose is detected in the urine, it is important to find out whether the underlying cause is benign or pathological.

Benign Glycosuria: In this category, may be placed "alimentary glycosuria," which is shown by some persons who, after eating a large amount of glucose or other carbohydrate, excrete glucose temporarily. "Emotional glycosuria" is another benign type of glycosuria. It is noted when a person is angry or is under nervous tension, for example while addressing an audience. In these conditions, there is also a transient hyperglycemia.

Renal glycosuria is also considered a benign type because the fasting blood sugar is normal. It appears to be due to the lowering of the renal threshold or the lowering of the blood-sugar level at which the kidneys will spill over glucose. The glycosuria that occurs in some pregnant women is thought to be a renal glycosuria.

Pathological Glycosuria: Pathological glycosuria is due chiefly to diabetes mellitus, which is apparently the result of a lack of insulin. It also occurs in hyperthyroidism, hyperpituitarism, increased intracranial pressure, hypertension, chronic liver disease, and acidosis of anesthesia or asphyxia. It is extremely important to differentiate diabetes mellitus from the other conditions mentioned above because of the difference in treatment.

Test for Glucose in Urine: Benedict's test is a commonly used procedure for the detection of reducing substances in the urine. The results are read as follows:

Clear blue	Negative for glucose
Pale green precipitate	Trace of glucose
Yellow precipitate	Up to 0.5 per cent
Brick red precipitate	Above 0.5 per cent
Brick red precipitate with clear supernatant fluid	1.5 per cent or more

False-positive tests may occur after taking salicylates, cinchophen, morphine, neocinchophen, phenacetin, antipyrine, and acetanilid.

Pentose:

Two types of pentosuria are recognized: (1) chronic pentosuria and (2) alimentary pentosuria. In chronic pentosuria, the output bears no relation to the amount of pentose in the food. It is apparently a harmless condition and bears no relation to diabetes mellitus. The cause of chronic pentosuria is not known.

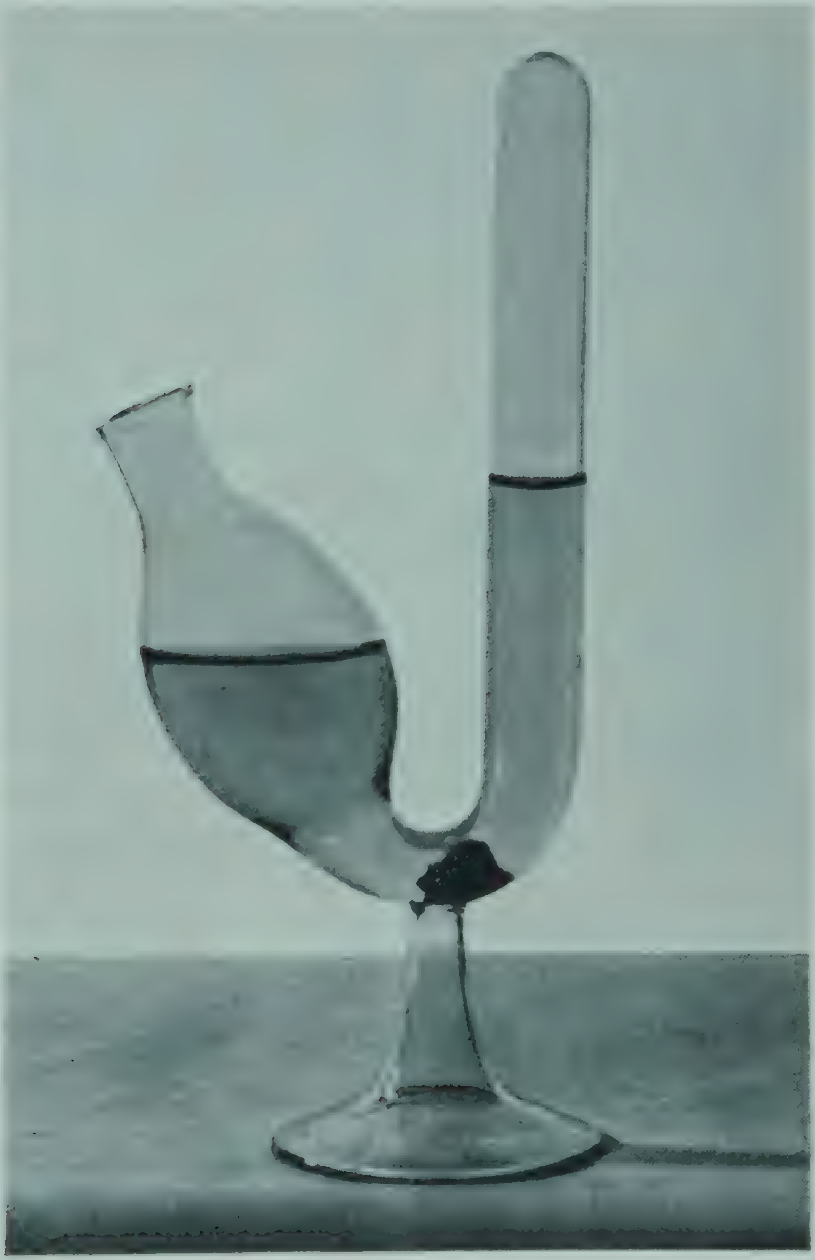

Fig. 3: Saccharometer, showing carbon dioxide in arm resulting from fermentation of glucose by yeast.

Alimentary pentosuria results from the ingestion of large amounts of fruits rich in pentose, such as prunes, grapes, cherries, plums, and fruit juices.

Pentosuria is of no great clinical importance except that it must be differentiated from diabetes mellitus.

Galactose:

This sugar has been found occasionally in the urine, particularly in that of nursing infants with deranged intestinal function. It is found also in cases of liver disease after administering galactose for a liver-function test (galactose-tolerance test).

Lactose:

Levulose, or fructose, occurs rarely in the urine. It is found after large amounts of the sugar. It is also found during pregnancy, during the nursing period, or shortly after weaning. It has no pathological significance except to differentiate it from glucosuria.

Levulose:

Levulose, or fructose, occurs rarely in the urine. It is found after the ingestion of fruits or honey, but it is transient. Essential levulosuria occurs, but it is very rare. Levulose is constantly excreted regardless of the amount in the diet. It is apparently a harmless metabolic abnormality and is of importance only to differentiate it from glucose before a patient is diagnosed as being diabetic.

PRODUCTS OF ACIDOSIS

Normally, fatty acids in the human body are completely oxidized to carbon dioxide and water, and intermediate products do not appear to any great extent in the blood or urine. In acidosis, ketone bodies accumulate in the blood (ketonemia) and are excreted in the urine (ketonuria). The ketone bodies are acetone, acetoacetic (diacetic) acid, and betahydroxybutyric acid.

Acetone:

Diabetes mellitus is perhaps the most important disease in adults in which acidosis develops with acetone excretion in the urine. Acetonuria also occurs in acidosis resulting from eclampsia, starvation, cachexia, digestive disturbance, febrile diseases in which carbohydrate intake is limited, cyclic vomiting of children, pernicious vomiting of pregnancy, and occasionally after chloroform and ether anesthesia.

Acetoacetic and Betahydroxybutyric Acids:

Acetone and betahydroxybutyric acid are derived from acetoacetic acid. Acetoacetic acid and betahydroxybutyric acid may occur in the urine as a result of the same diseases that cause acetonuria, but their presence is of more serious import.

The presence of appreciable amounts of ketone bodies in the urine was originally taken as an index of acidosis, the severity of which was determined by estimation of the levels of these substances in the urine. This is not a reliable index because acidosis occasionally occurs without any increase in ketone bodies. Thus, it becomes important to determine the alkali reserve in conjunction with the urinary ketone bodies.

PIGMENTS

Certain abnormal pigments are found in the urine under pathological conditions. These pigments are as follows:

Bilirubin
Urobilin
Urobilinogen
Hemoglobin
Porphyrins
Indican

Bilirubin:

Bilirubin is formed from hemoglobin by the reticuloendothelial system. Bilirubinuria occurs in partial or complete, intrahepatic and extrahepatic, obstructive jaundice. Bilirubin, which has passed through the liver (posthepatic), diffuses more readily than prehepatic bilirubin; consequently, the bilirubin of obstructive jaundice is found in the urine earlier than that of hemolytic jaundice. In fact, bilirubin may be absent in cases of hemolytic jaundice with a fairly high icteric index.

In infectious or toxic hepatitis and in cholangitis, as well as in partial obstruction of the biliary tract, bilirubin is present because the liver is unable to utilize the bilirubin absorbed from the intestinal tract plus the bilirubin formed by the reticuloendothelial system.

Detection of Bilirubin: Various tests have been devised for the detection of bilirubin in the urine. The best test appears to be the Harrison spot test, which is performed as follows:

1. Place from 5 to 10 cc. of urine in a test tube.
2. Add half that volume of 10 per cent aqueous barium-chloride solution and filter.
3. Open the filter paper with the precipitate of barium salts upon it and lay it on another dry filter paper.
4. Drop onto the precipitate one drop of reagent (trichloracetic acid 25 gm. plus 10 cc. of 10 per cent aqueous ferric chloride and 100 cc. of distilled water).
5. A green, or blue-green, color is indicative of the presence of bilirubin.

Urobilin (Stercobilin) and Urobilinogen:

When bilirubin enters the intestine, it is acted upon by bacteria, which convert it to urobilinogen. Then, by oxidation, urobilinogen is changed to urobilin. Most of the urobilin is excreted in the feces, some is destroyed, and some is returned to the liver by the portal system. If hepatitis is present, the bacteria can act upon the bilirubin in the liver and change it to urobilinogen. This creates an excessive amount in the bloodstream, and it is excreted in the urine.

Normally, there is only a trace of urobilin in the urine; but there is 1 to 4 mg. of urobilinogen excreted in twenty-four hours, which gives a positive test in a dilution of 1:20.

Urobilin and urobilinogen are increased in the urine in excessive hemolysis, as in hemolytic jaundice and pernicious anemia. In such cases, the liver may be functioning normally but is unable to take care of the large amounts of urobilin formed in the intestine; consequently, it passes into the circulation and is excreted by the kidneys. These two pigments are also increased in the urine in liver damage, such as toxic or infectious hepatitis, eclampsia, portal cirrhosis, decompensated heart, lobar pneumonia, malaria, and certain septicemias. A helpful differential diagnostic point is the fact that urobilinogen is rarely increased in obstructive jaundice unless it is accompanied by infection.

In diseases other than hemolytic anemias, increased amounts of urobilin and urobilinogen in the urine indicate that some bilirubin is entering the intestine and that the function of the liver is inadequate or infection is present in the liver.

Hemoglobin:

The presence of hemoglobin in the urine is called "hemoglobinuria," which is the result of excessive hemolysis. It occurs in malaria caused by Plasmodium falciparum (blackwater fever), after incompatible blood transfusions, in paroxysmal cold hemoglobinuria, paroxysmal nocturnal hemoglobinuria, march hemoglobinuria, paralytic hemoglobinuria, favism, and poisoning by mushrooms and certain chemical reagents.

The occurrence of hemosiderin crystals in the urine is called "urinary siderosis." It occurs in hemochromatosis, pernicious anemia, and kidney siderosis.[3]

Porphyrins:

The occurrence of porphyrins in the urine, together with certain clinical manifestations, is characteristic of a rare disease of pigment metabolism called "porphyria." According to Welcker,[4] all porphyrins have a basic porphyrin ring made up of four pyrrole groups attached to each other by methene linkages. The identity of a particular porphyrin is the result of type and position of radicals attached to eight substitution points along the porphyrin ring.

The porphyrins that are concerned in human metabolism and disease are protoporphyrin, uroporphyrin, and coproporphyrin. Protoporphyrin was first found to be an iron-free derivative of hemoglobin by Kammerer[5] in 1923 and was found in red cells by van den Bergh and Hyman[6] in 1928.

The porphyrias have been classified as "congenital," "acute," and "chronic." Nesbitt[7] feels that chronic porphyria may represent a mild form of congenital or acute porphyria.

Congenital porphyria is characterized clinically by photosensitivity, red teeth and bones, and dark-red or brown urine.[8] Type I isomers of uroporphyrin and coproporphyrin are found in large amounts in the urine of patients with this disease.

Acute porphyria is associated with acute episodes of abdominal cramps, neurogenic paralysis, mental symptoms, and dark urine.[7] The urine contains large amounts of Type III uroporphyrin and coproporphyrin. Protoporphyrin is not involved in either of these diseases, and no anemias have been found that would suggest an error in the formation of protoporphyrin. In fact, protoporphyrin has never been reported in the urine of hemoglobinuria or myoglobinuria.

Examination of the urine for uroporphyrin and coproporphyrin is carried out spectroscopically. It must be remembered that the zinc-metal complex of uroporphyrin forms absorption bands almost identical to those of oxyhemoglobin, and these bands are to be differentiated from them.

Watson and Schwartz[9] introduced a simple chemical test for porphobilinogen, which is positive in porphyria. The demonstration of a chloroform insoluble compound with Ehrlich's aldehyde reaction is pathognomonic for the disease.

In a test tube, mix equal parts of urine and Ehrlich's reagent. Add an equal volume of saturated sodium-acetate solution. Add a few cubic centimeters of chloroform, and mix thoroughly. The aldehyde compound of urobilinogen will be carried down by the chloroform; while porphobilinogen, which is insoluble in chloroform, will remain in the supernatant fluid, coloring it red.

According to de Langen and ten Berg,[10] lead poisoning may be detected before other signs appear because lead in the body increases the excretion of porphyrin to the point of fluorescence. The technic recommended by these authors is as follows:

A few drops of glacial acetic acid and 2 cc. of ether are added to 20 cc. of urine. The specimen is well shaken and exposed to ultraviolet rays through a Wood's glass.

If the concentration of porphyrin is normal, the layer of ether has a pale-blue or green light. Red, barely perceptible, with a slight excess of porphyrin becomes more intense with larger amounts.

Although slight degrees of porphyrinuria may be unnoticed, a false-positive reaction never occurs. When results are in doubt, the porphyrin may be estimated quantitatively. Pernicious anemia, hemolytic icterus, barbiturate poisoning, and other causes of porphyrinuria must be differentiated.

Indican:

The action of bacteria on tryptophane in the intestine produces indole, which is oxidized into indoxyl. Indoxyl is changed to indican (indoxyl potassium sulfate), presumably in the liver, and is excreted in the urine. Normally, the urine contains from 4 to 20 mg. in twenty-four hours.

Indicanuria is present in some healthy persons, some constipated persons, intestinal putrefaction resulting from intestinal obstruction, paralytic ileus, peritonitis, cholera, typhoid fever, achlorhydria, hyperchlorhydria (particularly with obstruction), diarrhea of pellagra, obstructive jaundice, and pernicious anemia. It is present also during decomposition of proteins, as in empyema, gangrene of the lung, pelvic abscess, and caseous tuberculosis with cavitation.

CALCIUM, CHLORIDES, AND PHOSPHATES

Calcium:

A person on an average diet excretes about 200 mg. of calcium in a twenty-four-hour output of urine. A decrease in urinary calcium is noted in tetany and, according to Robertson,[11] in myxedema. It is markedly increased in osteitis fibrosa cystica, although the diagnosis is usually made by x-ray examination of the bones and calcium, phosphorus, and phosphatase examination of the blood.

A rapid urine test for calcium is the Sulkowitch test, which gives an indication of the blood-calcium level and is of particular value in tetany. The test is carried out as follows:

1. Dissolve 2.5 gm. of oxalic-acid crystals and 2.5 gm. of ammonium-oxalate crystals in 145 cc. of distilled water, and add 5 cc. of glacial acetic acid (Sulkowitch reagent).
2. Mix equal parts of filtered urine and reagent in a test tube.
3. If no precipitate is present, the serum calcium is probably reduced to from 5 to 7.5 mg. per 100 cc. A firm, white precipitate indicates a normal serum-calcium level (from 9 to 11 mg. per 100 cc.). A milky precipitate indicates an increase above normal of serum calcium.

The test is best carried out on a twenty-four-hour specimen of urine with the patient on a calcium-low diet.

Chlorides:

With normal chloride intake and normal kidney function, from 10 to 16 gm. of sodium chloride are excreted in twenty-four hours. A decrease in urinary chlorides occurs when there is a decrease in blood chlorides, starvation, excessive sweating, vomiting, penumonia, ascites, pleuritic effusion, heart failure, nephritis, nephrosis, and burns.

In Addison's disease, there is increased urinary excretion of chloride in spite of decreased plasma chloride.

Phosphates:

Normally, about 70 per cent of ingested phosphate is excreted in the urine. This amounts to about 3 gm. in twenty-four hours. Of this, one third consists of amorphous phosphates (calcium and magnesium phosphates) and the remainder of alkaline phosphates (sodium and potassium phosphates).

Phosphates are increased in the urine in osteitis fibrosa cystica after taking parathyroid hormone, in alkalosis, and as a compensatory measure in acidosis to help maintain acid-base balance.

Phosphate elimination is decreased in nephritis with acidosis, in hypoparathyroidism with tetany, and in rickets.

SEDIMENT

A microscopic examination of the sediment after centrifuging should constitute a part of every routine examination of urine. Since the sediment changes on standing, it must be examined within five hours after voiding. If it must be kept longer, add four drops of formalin or five grains of boric acid for each four ounces of urine.

Sediment of Normal Urine

In normal urine, there may be a few desquamated epithelial cells, and rarely erythrocytes, leukocytes, and casts.

Unorganized sediments may be classified according to the reaction of the urine in which they are most likely found. Although, this grouping is useful, it is not always accurate, since the characteristic sediments of acid urine may remain after the urine has become alkaline, and also the alkaline sediments may be precipitated in a urine that is still acid. The following may be found in acid urine: uric acid, sodium urate, calcium oxalate, primary calcium phosphate, and ammoniomagnesium phosphate. An alkaline urine may contain secondary calcium phosphate, tricalcium phosphate, calcium carbonate, ammoniomagnesium phosphate, and ammonium biurate.

Spermatozoa are sometimes found in the normal male urine following sexual intercourse, nocturnal emissions, convulsions, and prostatic massage.

SEDIMENT OF PATHOLOGICAL URINE

Epithelial Cells:

An increase in the number of epithelial cells is due to a pathological process at the site of exfoliation. The small, round or polygonal cells are from the tubules; and when present in casts, they suggest nephrosis. Spindle-shaped cells are from the kidney pelves, ureters, or bladder. Large, polygonal, squamous cells are from the bladder or vagina from contaminating vaginal discharge (leukorrhea) .

Recent studies resulting from the work of Papanicolaou indicate that tumors of the kidney may be diagnosed by those who are familiar with kidney cystology.

Erythrocytes:

After ruling out contamination by menstrual blood and catheter trauma, the presence of more than an occasional erythrocyte is considered pathological. By the method of Addis, they may vary from 0 to 425,000 in a twelve-hour specimen. Hematuria occurs in glomerular nephritis; infarction; tuberculosis of kidneys; pyelonephritis; tumors (particularly carcinomas) of kidneys, ureters, and bladder; polycystic kidneys; calculi; and hemorrhagic diseases, such as purpura haemorrhagica and hemophilia. Hematuria has occurred following dicumarol therapy of thrombophlebitis, phlebothrombosis, and so on.

Red cells are not found in the urine in lipoid nephrosis. This helps to differentiate it from acute nephritis.

Leukocytes:

A few leukocytes may be found in normal urine. Extraneous leukocytes from leukorrhea in the female must be ruled out. Leukocytes may be numerous enough to give the urine a turbid appearance.

Increased numbers of leukocytes are found in urethritis, cystitis, prostatitis, pyelitis, pyelonephritis, seminal vesiculitis, tuberculosis of the kidneys, and following trauma of catheterizing the urethra or ureters. It is difficult to determine whether the leukocytes originate in the bladder, ureters, or kidneys. Cystoscopy helps to determine the source by collection of specimens from the bladder and from each kidney.

CASTS

Casts are cylindrical structures that form in the kidney tubules by the coagulation of protein. The occurrence of casts is called "cylindruria."

Noncellular Casts:

The noncellular casts are hyaline, granular, waxy, and fatty in type.

Hyaline casts are composed of a protein compound (chondroitin sulfuric acid), which is apparently derived from the tubules. When these casts are wide, it indicates exfoliation of the tubular epithelium and is of more serious import. Hyaline casts may occur in the various types of glomerular nephritis, nephrosis, arteriosclerotic nephritis, and febrile conditions that bring about cloudy swelling of the tubular epithelium.

Granular casts are either finely or coarsely granular and are apparently of the same composition as hyaline casts. Their presence suggests nephrosis or some type of glomerulonephritis.

Waxy casts may occur in glomerulonephritis as well as in renal amyloid disease.

Fatty casts are characterized by the presence of fat droplets, which are embedded in hyaline casts. They apparently form as a result of fatty degeneration of tubular or glomerular epithelium. They are found in lipoid nephrosis and chronic glomerulonephritis.

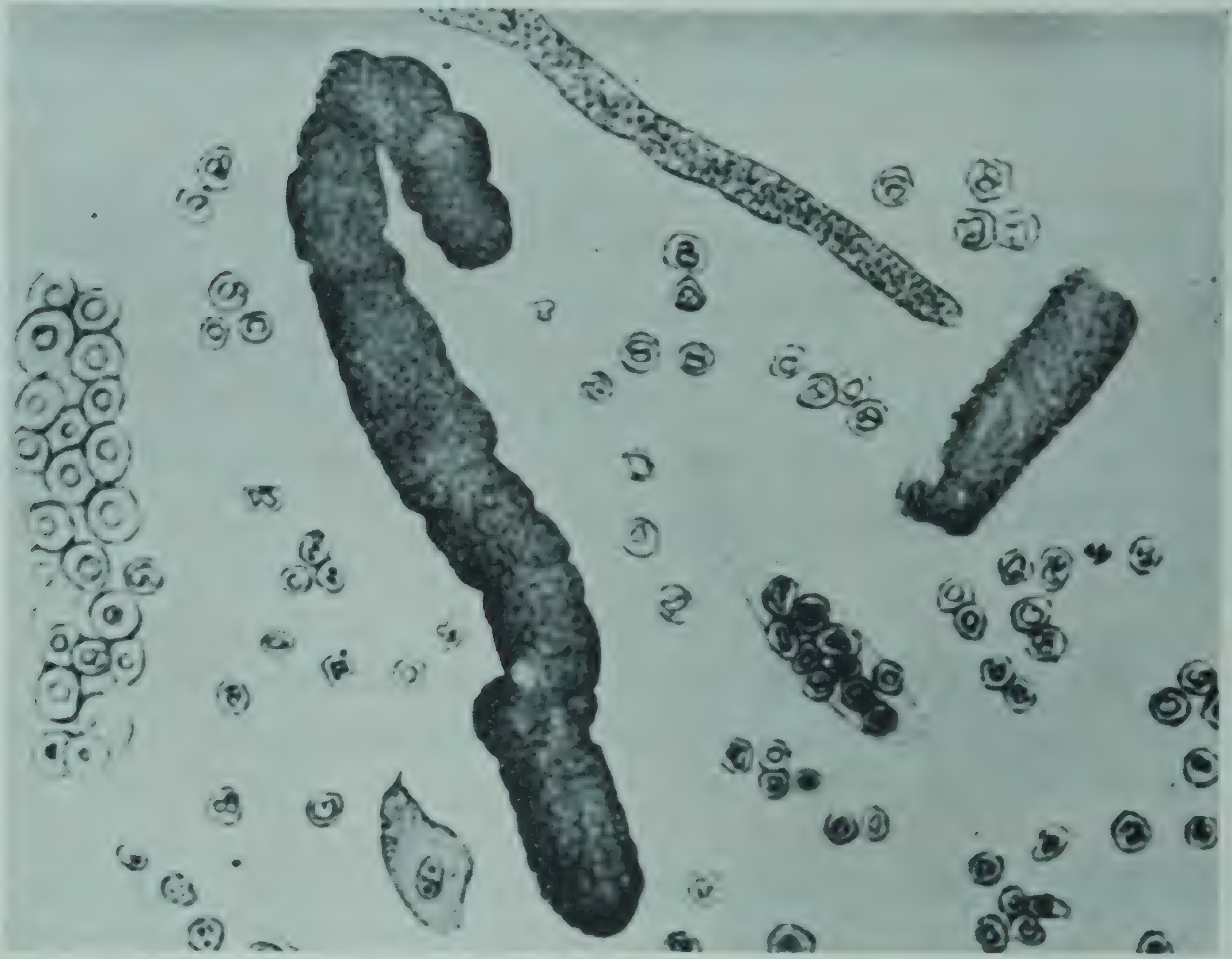

Fig. 4: Casts of urine sediment: white-cell cast, granular casts, and pus cells.

Cellular Casts:

Leukocyte casts made up of white cells indicate pyelonephritis.

Red-cell casts are formed as a result of hemorrhage from the glomeruli. Since plasma passes out with the red cells, the formation of casts is not difficult. They may consist of well-formed erythrocytes or mere shadows as a result of hemolysis. They are found in chronic glomerulonephritis and following incompatible transfusions resulting in hemolysis.

Epithelial casts are made up of degenerated epithelial cells from the tubules. They occur in nephrosis, as a result of toxins, in febrile diseases, eclampsia, poisoning by heavy metals, and amyloid disease.

So-called "renal-failure" casts occur at the lower ends of the tubules and are made up of debris. They occur suddenly, particularly with a large output of urine after a period of oliguria.

Bacterial casts are rare; and when seen, indicate a septic condition of the kidney.

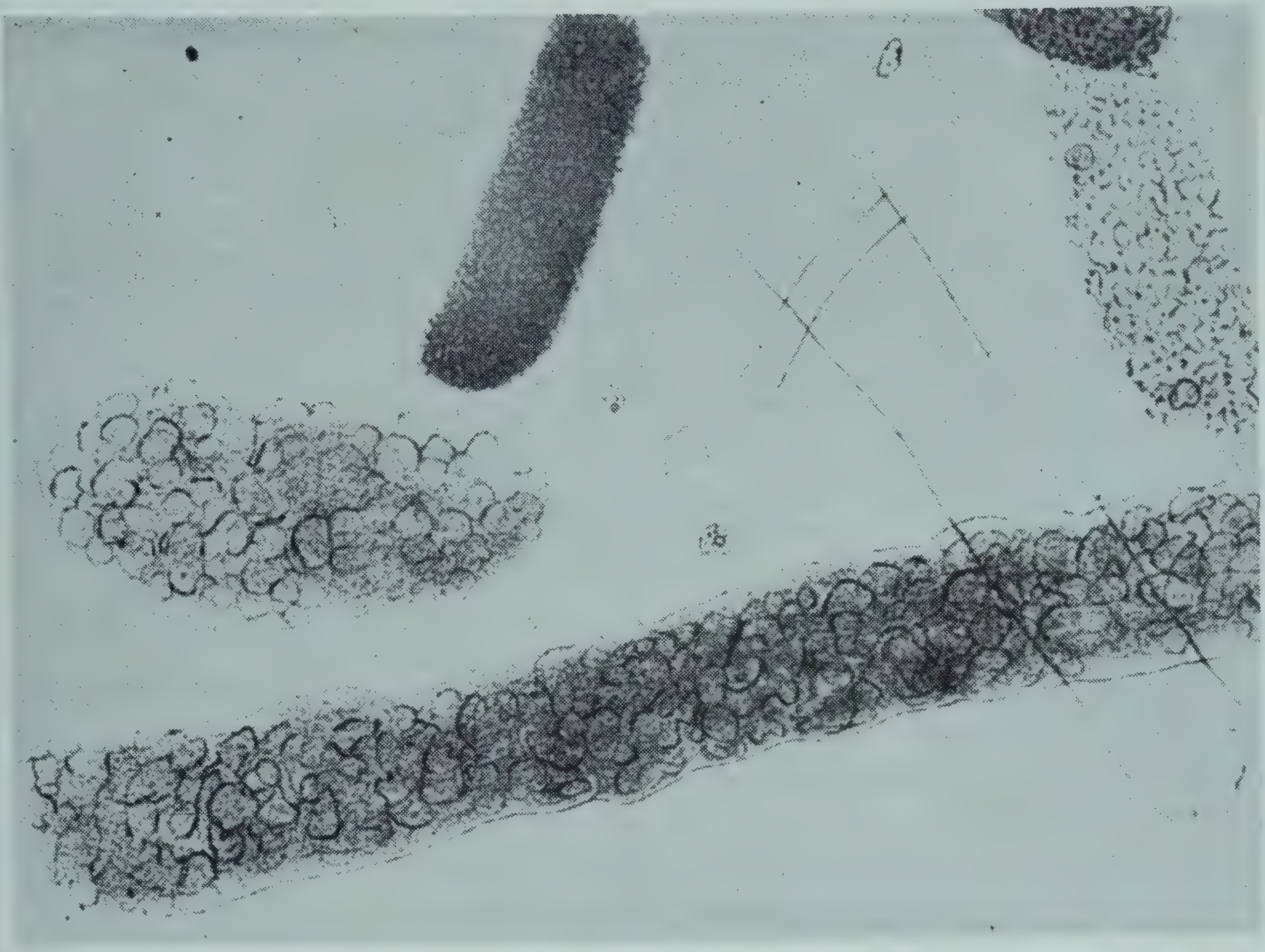

Fig. 5: Casts of urine sediment: epithelial-cell casts and white-cell cast.

Structures Similar to Casts:

Mucous threads and cylindroids may be confused with casts. Cylindroids resemble hyaline casts but differ in that they taper to a slender tail, which may be curled. Hairs, fibers, and hyphae of fungi can usually be differentiated from casts by careful study.

ADDIS COUNT

Addis,[12] and Addis and Oliver[13] have described a method of enumerating casts and cells in a twelve-hour sample of urine. They state that there is an appreciable number of these elements in normal urine, but wide variations occur in different types of nephritis.

Procedure:

1. Save a twelve-hour sample of urine, which must be measured accurately within ±2 cc.
2. Transfer 10 cc. of urine to a special, graduated, centrifuge tube. The narrow tip is graduated for the measuring of small amounts of sediment.
3. Centrifuge for five minutes at 1800 r.p.m.
4. Pour off the supernatant urine, and adjust the volume of the remaining fluid with salt solution (1 to 5 cc.). The sediment from the 10 cc. of urine can then be well distributed in the fluid by mixing thoroughly with a fine pipet.
5. Place a drop of the mixture of sediment in a hemocytometer, and count the number of casts in the total ruled area. This represents the number in 0.0009 cc. Also count the erythrocytes and the leukocytes that are found in the same ruled area. Repeat this procedure two to ten times and add the number of cells and casts that have been counted.

Calculation:

The following formula is self-explaining: Let V = volume of urine (expressed in cubic centimeters) for twelve hours; 10 = number of cubic centimeters of urine centrifuged; s = volume in cubic centimeters of mixed sediment; v = volume in cubic centimeters in which count was made; n = total number of cells or casts counted; and N = number of casts or cells in a twelve-hour sample.

$$N = \frac{s}{v} \times n \times \frac{V}{10}$$

Interpretation:

Hyaline casts may normally number as high as 5000 in the twelve-hour period; but in nephritis, casts of various sorts may total from 50,000 to 1,000,000. Erythrocytes normally number from 0 to 500,000 (possibly even 1,000,000). In nephritis, there may be an enormous increase in the number of erythrocytes in the twelve-hour period (from 15 to 400,000,000). Leukocytes normally may be present and usually do not exceed 1,000,000 in twelve hours. In pathological conditions, the epithelial cells and pus cells may number from 2,000,000 to 50,000,000. It is evident that with such wide variations in normal persons a close division between normal and abnormal is impossible.

CRYSTALS

Leucine and Tyrosine:

These crystals occur in the urine as a result of marked liver damage, such as fatty degeneration, acute yellow atrophy, and poisoning by chloroform, phosphorus, and carbon tetrachloride. Tyrosine crystals have also been observed in necrotizing carcinoma of the lung and sloughing skin lesions.

These crystals are cleavage products of the protein molecule, and some believe that they are the result of incomplete deamination by the liver.

Leucine crystals are yellow, oily-appearing spheres, many with radial striations and concentric contours. Tyrosine occurs after crystallization as a black mass of fine needles arranged in sheaves with a central constriction.

Calcium Oxalate:

These crystals may occur in large numbers in the presence of a calculus and in certain digestive upsets.

Uric Acid:

Excess uric-acid crystals in fresh urine suggest the presence of a calculus or abnormal uric-acid metabolism, as in gout.

Sulfonamides:

Crystal forms of certain derivatives of sulfanilamide may precipitate out in the urine. These crystals should be recognized and reported to the physician when they are present. They are significant because of the possibility of the formation of renal calculi or deposition in the tubules with subsequent hematuria.

BACTERIOLOGICAL EXAMINATION

The bacteriological examination of urine will be discussed in Chapter 13 (see p. 255).

OVA AND PARASITES

Trichomonas vaginalis and ova of Oxyuris vermicularis are sometimes washed from the vagina and are found in the urine sediment. The ova of Schistosoma haematobium are also found in the urine, particularly in Egypt. The larvae of filariae have been found in countries where filariasis exists. Scolices and hooklets of hydatid cysts constitute a rare finding in the urine.

Yeasts, fat droplets, starch granules, and dusting-powder granules must not be confused with ova.

SPECIAL TESTS

Amylase:

A small amount of the starch-splitting ferment, amylase or diastase, is present in normal urine. The amount of amylase is increased above 300 units by the Fabricius-Møller[14] test in the first forty-eight hours of acute pancreatitis, in obstruction of the common duct by a gallstone, and sometimes in perforating peptic ulcer. It is decreased in liver necrosis and in severe burns.

Pregnancy Tests:

In 1928, Aschheim and Zondek introduced a successful urine test for pregnancy based on the formation of an anterior-pituitary-like hormone, presumably by the trophoblasts of the placenta. These authors used five or six mice in the original test, but this has been simplified by Friedman,[15] who used a single, virgin, female rabbit. After injecting from 10 to 15 cc. of filtered morning urine made slightly acid, the ovaries are examined in twenty-four hours, and preferably again after another twenty-four hours. The presence of bulging hemorrhagic follicles indicates a positive test.

If the patient is bleeding vaginally, she should be catheterized because human blood will kill a rabbit.

The test is said to be positive one to two weeks after conception, but practically it is not reliable until one week after the first missed menstruation. In addition to normal uterine pregnancy, it is positive in about 50 per cent of ectopic pregnancies, missed abortion,

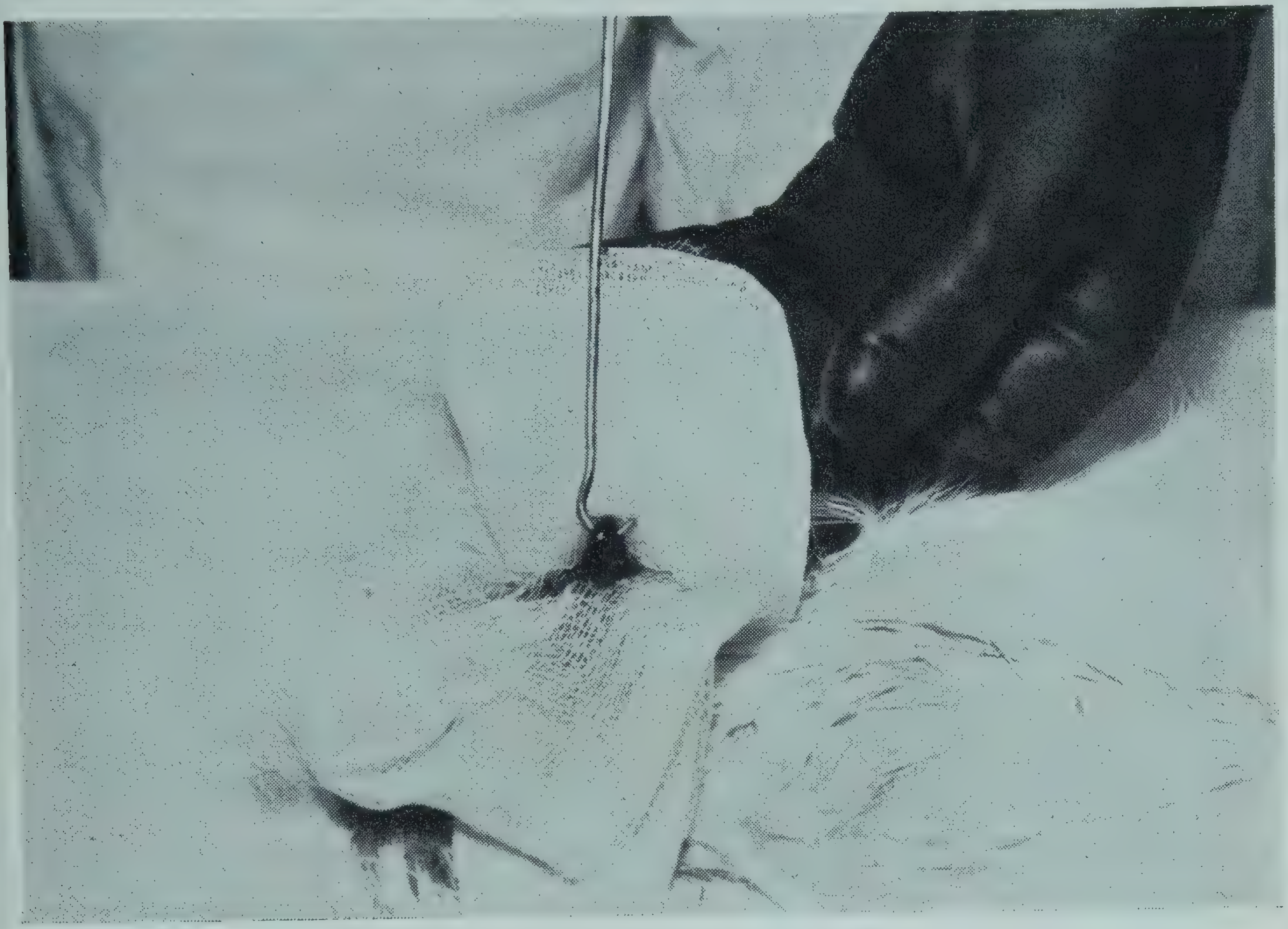

Fig. 6: Demonstration of ovary of rabbit in Friedman test for pregnancy.

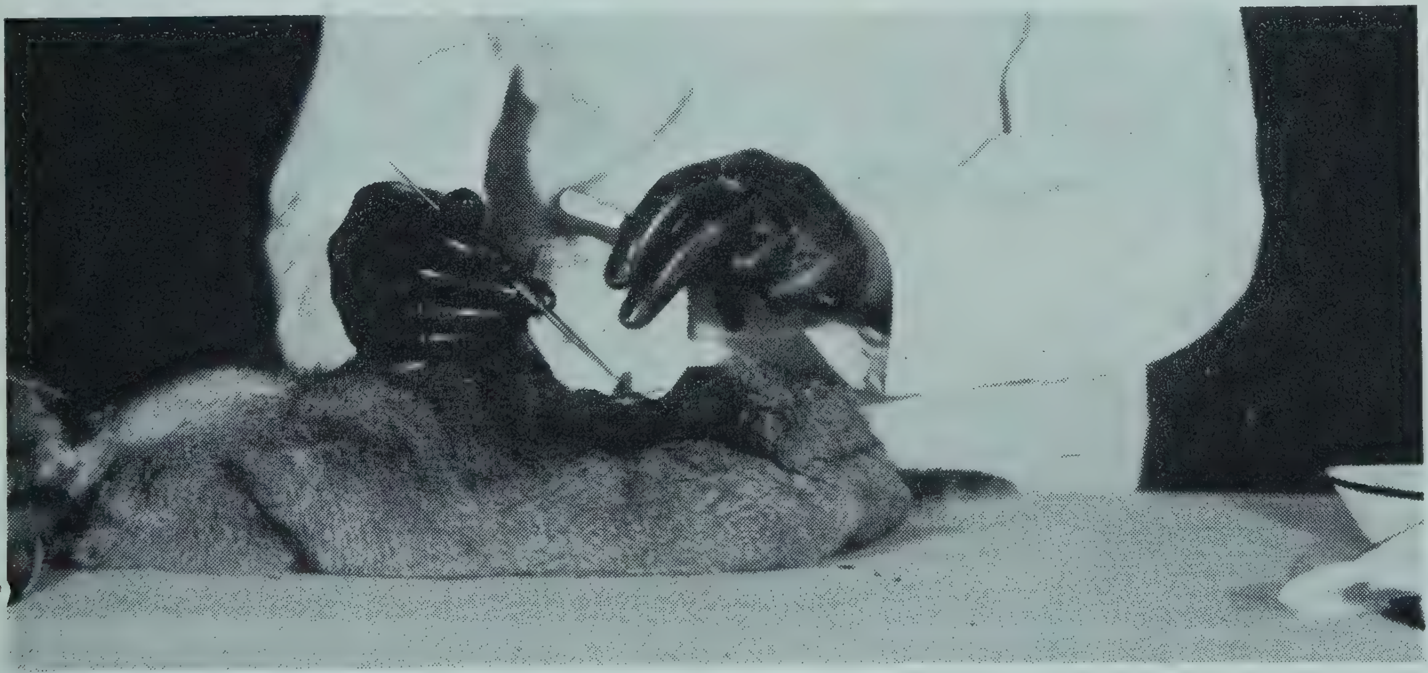

Fig. 7: Demonstration of rabbit ovary exposed through back incision (Friedman test).

and incomplete miscarriages as long as viable placenta is in biological contact with the maternal circulation. Certain tumors also cause a positive Aschheim-Zondek or Friedman test. They are hydatidiform mole, chorioma of the placenta, and chorioma of the ovary and testicle.

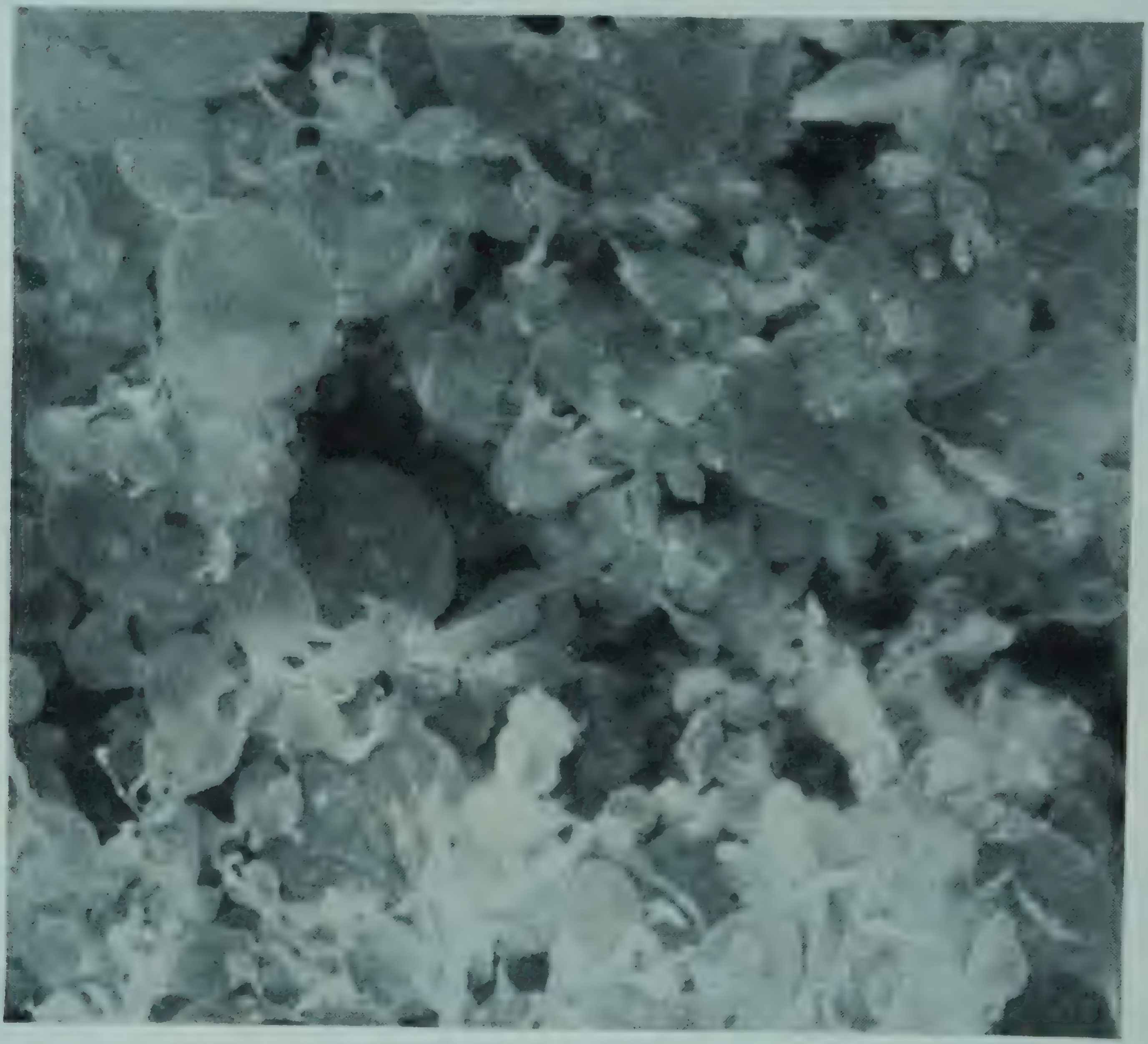

Fig. 8: Hydatidiform mole.

In view of the excessive amounts of trophoblast in hydatidiform mole and chorioma, increased amounts of gonadotropic hormones are found in the blood and urine. Such cases normally contain at least 200,000 mouse units per liter of urine, although some cases have shown less than this. Zondek lays stress upon the use of cerebrospinal fluid in the above tumors. It gives a negative test in normal pregnancy but is positive in placental tumors and in chorioma of the ovary and testicle.

The Friedman test becomes negative seven to ten days after miscarriage or death of the fetus, five to ten days after delivery, and after cure of a hydatidiform mole. It is advisable to do follow-up pregnancy tests every three months for a year after the delivery of a hydatidiform mole to make sure that there are no remaining fragments that may become malignant (chorioma).

False-negative tests may occur in 1 to 2 per cent of tests. They may also occur in rabbits too young to produce a reaction. False-positive reactions may rarely occur in early menopause, certain ovarian cysts, adenocarcinoma of the uterus, hyperplasia of endometrium, hyperthyroidism, and pituitary compensation associated with ovarian failure. These reactions are usually slight and are characterized usually by ripening of the follicles without hemorrhage.

The use of frogs has shortened the time for the pregnancy test. With the female Xenopus laevis, the test can be completed in twelve hours; and with the male Rana pipiens, it takes only three hours. These modifications check favorably with the rabbit test.

KIDNEY-FUNCTION TESTS

Kidney-function tests, applicable to the kidneys and urinary excretion, fall into three categories:

1. The ability of the kidneys to excrete certain chemicals, such as phenolsulfonphthalein
2. The ability of the kidneys to excrete urea (urea clearance) and creatinine (creatinine clearance)
3. The ability of the kidneys to concentrate and dilute urine (concentration and dilution tests)

The examinations of the blood for nitrogen retention in kidney disease have been discussed in Chapter 2 (see p. 53).

EXCRETION TESTS

Phenolsulfonphthalein Test:

This test was introduced by Rowntree and Geraghty in 1910 and consists in the intramuscular or intravenous injection of a solution of phenolsulfonphthalein, which is excreted by the kidneys. The amount eliminated within a certain time period is indicative of the excretory capacity of the kidneys. The use of a catheter is not necessary unless there is obstruction.

Procedure:

1. Give the patient about two glasses of water.
2. Twenty minutes later, have the patient empty the bladder and discard the urine.
3. Inject 1 cc. of sterile phenolsulfonphthalein solution intramuscularly or intravenously. (The latter is preferred if there is generalized edema.)
4. One hour and ten minutes later, have the patient empty the bladder and save the urine. (The ten-minute period represents the usual time that elapses between the injection and the first appearance of the dye in the urine.)
5. One hour after this or two hours and ten minutes after the injection have the patient empty the bladder, and save the urine in a separate container.
6. Estimate the amount of phenolsulfonphthalein in each specimen and report as per cent.

Interpretation:

In the first hour, the amount of dye excreted is normally from 40 to 60 per cent. In two hours, it is from 55 to 80 per cent. When the total excretion is below 55 per cent, it is considered abnormal. A low excretion in the first hour and a compensatory increase in the second hour, making the total above 55 per cent, are abnormal.

The output is reduced in chronic nephritis, heart failure, cirrhosis with normal renal function, generalized edema, and sometimes urinary tract obstruction. It may be increased in cases of hyperthyroidism, hypertension, hepatic damage, and nephritis with increased urinary output.

Phenolsulfonphthalein Test of Two Kidneys Separately:

Procedure:

1. The dye (1 cc.) is given after the ureteral catheters are in place.
2. The urine is received directly from the catheters into two test tubes that contain a few drops of 10 per cent sodium hydroxide.
3. The time of the first appearance of the dye is noted.

4. The urine is collected for one hour, in fifteen-minute periods.
5. The amount of dye in each specimen is computed in per cent.

Interpretation: The dye first appears in from three to five minutes after intravenous injection. The output for the two kidneys is as follows: fifteen minutes from 35 to 45 per cent; half hour, from 50 to 60 per cent; one hour, from 65 to 80 per cent.

When one kidney is not functioning properly, the appearance of the dye from that kidney is delayed, and the total excretion from that kidney is reduced. The other kidney may compensate in part by an increased output of dye. The bladder should be checked to make sure that no dye has leaked past the catheters.

CLEARANCE TESTS

Urea-Clearance Test:

This test indicates the ability of the kidney to excrete urea. It is preferable to carry out this test in the morning without breakfast.

Procedure:

1. The patient is given a glass of water and is instructed to empty the bladder.
2. The specimen is discarded, but the time of passing the urine is noted.
3. At the end of one hour, the patient is instructed to void again, and the time is noted. This specimen is saved for laboratory analysis.
4. At about this time, a sample of blood is taken in potassium oxalate for determination of urea nitrogen.
5. A second glass of water is given.
6. One hour later, the bladder is emptied, and the time is recorded. The volume of this specimen in cubic centimeters is divided by 60 to calculate the amount per minute (V).
7. The blood and two specimens of urine are sent to the laboratory with the time recordings as indicated.

Calculation:

1. Dilute 5 cc. of urine to 50 cc. with distilled water, and determine the amount of urine urea nitrogen plus ammonia nitrogen in 5 cc. of diluted urine (0.5 cc. of undiluted urine).
2. Determine the amount of ammonia nitrogen in 5 cc. of diluted urine. Subtract the value for ammonia nitrogen from the value for urine urea plus ammonia nitrogen, which gives the amount of urine urea nitrogen.

3. The urea clearance equals the number of cubic centimeters of blood cleared of urea per minute. If the quantity of urine voided is more than 2 cc. per minute, the formula used is that for "maximal clearance" (Cm):

$$\frac{U}{B} \times V = \text{cubic centimeters of blood cleared of urea per minute}$$

U = urine urea nitrogen, milligrams per 100 cc.
B = blood urea nitrogen, milligrams per 100 cc.
V = cubic centimeters of urine excreted per minute

4. If the amount of urine voided is less than 2.1 cc. per minute, the square-root law is applied, and the formula for "standard clearance" (C_s) is used:

$$\frac{U}{B} \times \sqrt{V} = \text{cubic centimeters of blood cleared of urea per minute}$$

The square root values are in Table 5.

Table 5: Square-Root Values for Number of Cubic Centimeters per Minute

V	$\sqrt{V}$	V	$\sqrt{V}$	V	$\sqrt{V}$	V	$\sqrt{V}$
0.2	0.45	0.7	0.84	1.2	1.10	1.7	1.30
0.3	0.55	0.8	0.89	1.3	1.14	1.8	1.34
0.4	0.63	0.9	0.95	1.4	1.18	1.9	1.38
0.5	0.71	1.0	1.00	1.5	1.23	2.0	1.42
0.6	0.78	1.1	1.05	1.6	1.27	2.1	1.45

Interpretation: The normal for maximum clearance is considered to be 75 cc., while that for standard clearance is 54 cc. It is decreased in acute nephritis, active chronic glomerulonephritis, arteriosclerosis with hypertension, congestive heart failure, and eclampsia with permanent kidney damage.

Other Clearance Tests:

Creatinine, insulin, and diodrast have been used for clearance tests, but at present there is no reason to believe that any one of them will replace the urea clearance. The normal clearance for these is as follows:

Creatinine: 148 (±34) cc. of plasma per minute[16]
Inulin: 125 cc. of blood per minute[17]
Diodrast: 700 cc. of blood per minute[17]

CONCENTRATION AND DILUTION TESTS

The normal kidneys excrete urine that differs markedly in volume and specific gravity at different periods within the twenty-four hours. These variations are in response to the varying demands for the elimination of water and solids to keep the body fluids constant in spite of periodic food and fluid intake. Damaged kidneys lose partially or completely this ability to respond to the needs of the body, with the result that the urine has almost the same specific gravity throughout the day.

Mosenthal-Test-Meal Concentration Test Procedure:-

Procedure:

1. On the day of the test, and preferably the day before, place the patient on a full diet. At least a pint of fluid, including water, tea, or coffee, is taken at each meal. No food or liquid is to be taken between meals or until 8 A.M. the following morning (day of the test).
2. Instruct the patient to empty his bladder before breakfast.
3. Collect urine in separate containers every two hours from 10 A.M. to 6 P.M. (six specimens).
4. Collect all urine in one container between 8 P.M. and 8 A.M. of the following morning.
5. Measure and take the specific gravity of all specimens. If albumin is present, subtract 0.003 from the specific gravity for each gram of albumin per 100 cc.

Interpretation: In the normal person, the night urine will be much less than the total day urine, usually not over 300 cc. The highest specific gravity will be higher than 1.018, and the difference between the highest and lowest will not be less than eight points.

In kidney disease, the night urine will exceed 750 cc. This is usually one of the first evidences of impaired kidney function. The specific gravity is lower than 1.018. Fixation of specific gravity with a variation of only one or two points is indicative of impaired kidney function. It becomes fixed at a lower level as the impairment of function increases.

Sometimes, fixation of specific gravity occurs near 1.018 or 1.020 in acute nephritis and chronic passive congestion of the kidneys. It may, however, result from insufficient fluid intake or excessive sweating. The excretion of edema fluid during the test may cause fixation of specific gravity, and the results will be confusing.

Volhard's Concentration Test Procedure:

Procedure:

1. Allow no food between meals and no fluid from the evening before.
2. Give breakfast at 8 A.M. of dry cereal with sugar or syrup, no milk, one egg, and toast or bread with butter.
3. Give dinner at 12 noon of roast beef, steak, or chops, boiled or baked potatoes, and bread with butter or jam.
4. Give supper at 5 P.M. of two eggs and bread with butter or jam.
5. At 8 A.M. of the same day, empty the bladder and collect urine every three hours, that is at 11 A.M., 2 P.M., 5 P.M., and 8 P.M. The next morning, collect all urine from 8 P.M. to 8 A.M.
6. Note the quantity and specific gravity of each specimen.

Interpretation: In normal persons, the specific gravity should be at least from 1.025 to 1.030. In severe kidney disease, the specific gravity may be fixed near 1.010.

Fishberg's Concentration Test Procedure:

Procedure:

1. The patient may eat a regular meal at 6 P.M. with a minimal amount of fluid and considerable protein.
3. Discard all urine passed during the night.
3. Save the first specimen passed in the morning.
4. Keep the patient in bed for one hour after waking, and collect a second specimen in a separate container.
5. If possible, have patient arise, and collect a third sample after an hour of activity.
6. Send the three samples to the laboratory for determination of specific gravity.

Interpretation: In normal persons, the specific gravity should range between 1.022 and 1.032; in cases of severe kidney disease, the specific gravity will be as low as 1.010; and in conditions of less severity, it will range between 1.010 and 1.020.

Fishberg's Dilution Test Procedure:

Procedure:

1. On the day of the test, omit breakfast. Give the usual diet for dinner and supper with one glass of water after supper.

2. Have patient empty the bladder, and discard specimen at 8 A.M. Give 1500 cc. of water.
3. Collect urine at half-hour intervals, beginning at 9 A.M. and ending at 12 noon Collect urine from 12 noon until 8 A.M. the next day in one container.
4. Measure volume, and take the specific gravity of each specimen.

Interpretation: The normal first-hour specimen is about 40 cc., with a specific gravity of from 1.001 to 1.003. After this, the volume decreases, and specific gravity increases until the fourth hour when the volume is 100 cc. and specific gravity is 1.012 to 1.016. The total volume excreted should be from 80 to 120 per cent of intake (from 1200 to 1800 cc.).

The test should not be conducted when the patient is dehydrated or edematous since results will not be reliable.

In persons with impaired renal function, the output in the first hour is reduced to 200 cc. or less, and the specific gravity is 1.010 or higher.

DRUGS AND POISONS

This subject is discussed in Chapter 17 (p. 315).

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CHAPTER 5

Gastric and Duodenal Contents

PHYSIOLOGY OF THE STOMACH

If the various functions of the stomach are analyzed, they fall into the following categories:

1. Temporary storage place for food and fluid.
2. Production of pepsin, rennin, lipase, mucin, and hydrochloric acid, and initiation of digestion: Pepsin is secreted as pepsinogen and initiates the breaking-up of proteins. Renninogen is secreted by the chief cells in the glands of the fundus and is changed to rennin. This enzyme coagulates milk. Lipase is a weak, fat-splitting enzyme, which is active in the hydrochloric acid of the stomach. Mucin serves as a coating for the mucosa and also neutralizes hydrochloric acid. Hydrochloric acid is thought to be secreted by the parietal cells and serves as a medium in which pepsin can best digest proteins. It is also a mild antiseptic and thus acts to prevent putrefactive processes.

   Although there is no specific enzyme for the digestion of sugar in the stomach, it is slowly split up into levulose and glucose. This process is due to the action of acid.
3. Absorption of water, alcohol, and simple compounds: In addition to water and alcohol, such simple compounds as glucose, peptone, and sodium chloride are absorbed in part or completely.

4. Formation of an antianemic factor: The experimental work of Castle and his colleagues[1] has been outstanding in explaining the factor that is lacking in the gastric juice of patients having pernicious anemia. The theory is based upon the results obtained by feeding patients having pernicious anemia mixtures of normal gastric juice and beef, which had been incubated together for two hours at 98.6° F. (37° C.) at *p*H 7. He also demonstrated that feeding of gastric juice alone or beef alone gave no improvement in pernicious anemia. The mucosa of pig stomach incubated with beef also gave beneficial results, thus demonstrating that the agent in the normal gastric juice was previously in the gastric mucosa. These experiments led Castle to evolve the theory of an intrinsic and extrinsic factor. The active constituent (intrinsic factor) of the normal, human, fasting, gastric contents is, in all probability, secreted by the stomach. It is capable of interaction with protein (extrinsic factor) or closely related substances in neutral solution, resulting in the production of an agent having a marked hemopoietic effect when administered to patients having pernicious anemia.

NORMAL GASTRIC CONTENTS

Normal fasting gastric juice is a limpid, light-colored fluid, which is acid in reaction and has a specific gravity of 1.007. It contains about 0.5 per cent of solid matter, which is made up mostly of sodium chloride, potassium chloride, earthy phosphates, mucin, and the enzymes, pepsin, rennin, and lipase. The acidity of the gastric juice is caused by the presence of free hydrochloric acid amounting to from 0.4 to 0.6 per cent.

In addition to the free hydrochloric acid, there is the total acidity. This consists of free hydrochloric acid, combined hydrochloric acid, acid salts, and, when present, organic acids.

The following is a summary of the findings in normal gastric juice with the patient fasting:

Color: Yellow (pale green if bile has been regurgitated)
Volume: 20 to 100 cc.
Blood: none
Bacteria: a few similar to those in mouth and throat; most are dead if there is a normal amount of hydrochloric acid
Free hydrochloric acid: expressed in terms of the number of cubic centimeters of decinormal sodium hydroxide required to neutralize 100 cc.; it averages 18 degrees

Total acidity: (free hydrochloric acid, hydrochloric acid combined with protein, and acid salts): 10 to 50 degrees. Organic acids (lactic acid, butyric acid, and other fatty acids): not present
Enzymes (pepsin, rennin, and lipase): present
Mucus: small amount

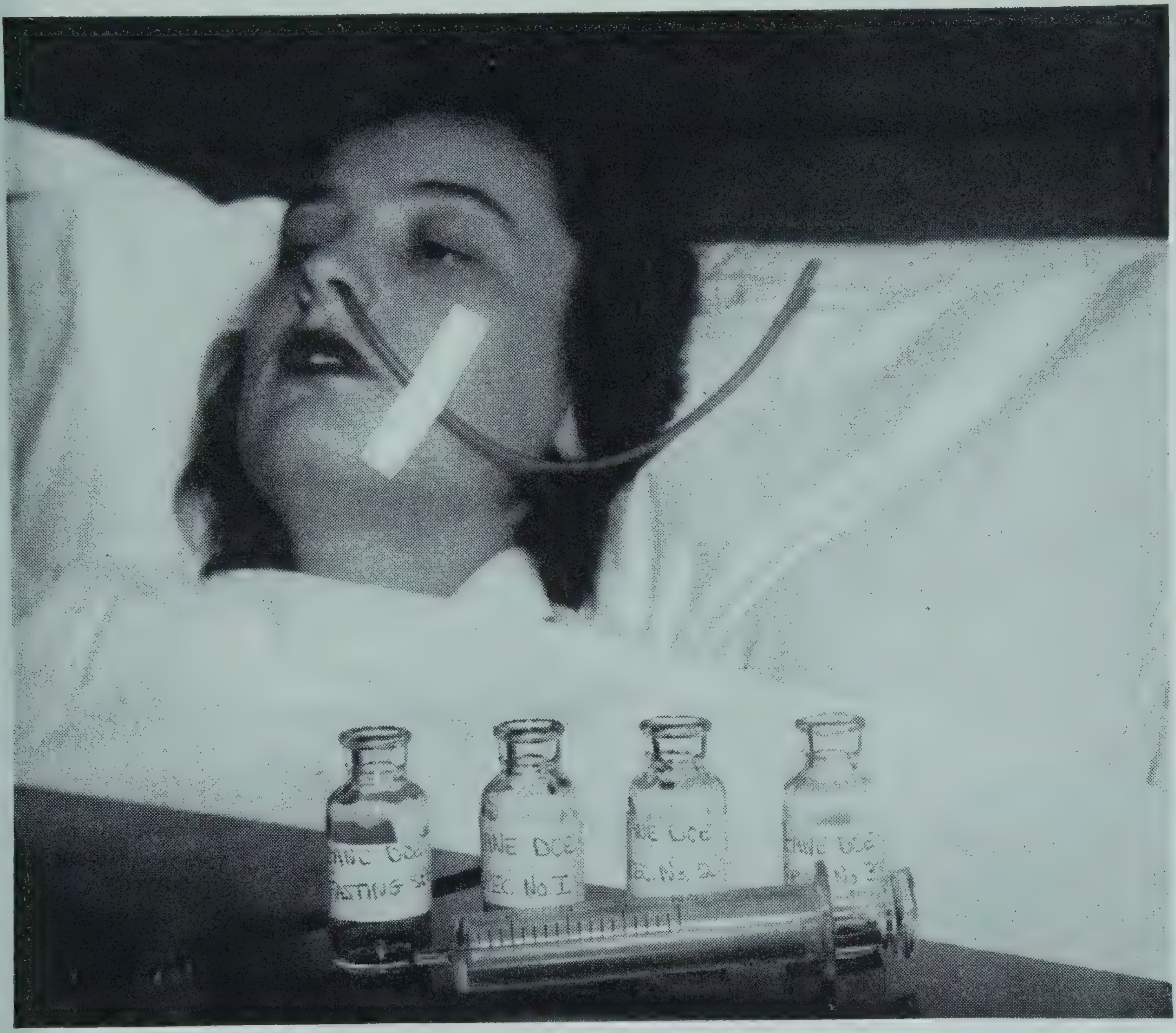

Fig. 1: Patient prepared for fractional gastric analysis.

Normal Response to Test Meal:

In order to determine the amount of hydrochloric-acid production, various test meals have been devised. It is advisable to give the test meal in the morning when the stomach is normally empty.

1. Ewald's test meal: This consists of a roll or two slices of bread without butter, and two small cups of water or weak tea without cream or sugar.
2. Alcohol test meal: This consists of 50 cc. of 7 per cent ethyl alcohol injected through the gastric tube.

3. Histamine phosphate test: With the patient fasting for at least twelve hours, administer hypodermically 0.25 mg. of histamine phosphate. Because of the reaction that may follow, care must be exercised in the choice of patients.
4. Fractional Method: When it was appreciated that there was great variation in the time at which the height of digestion was reached, the fractional method came into use:
   a. Rehfuss or other tube is inserted into the stomach, and it is emptied.

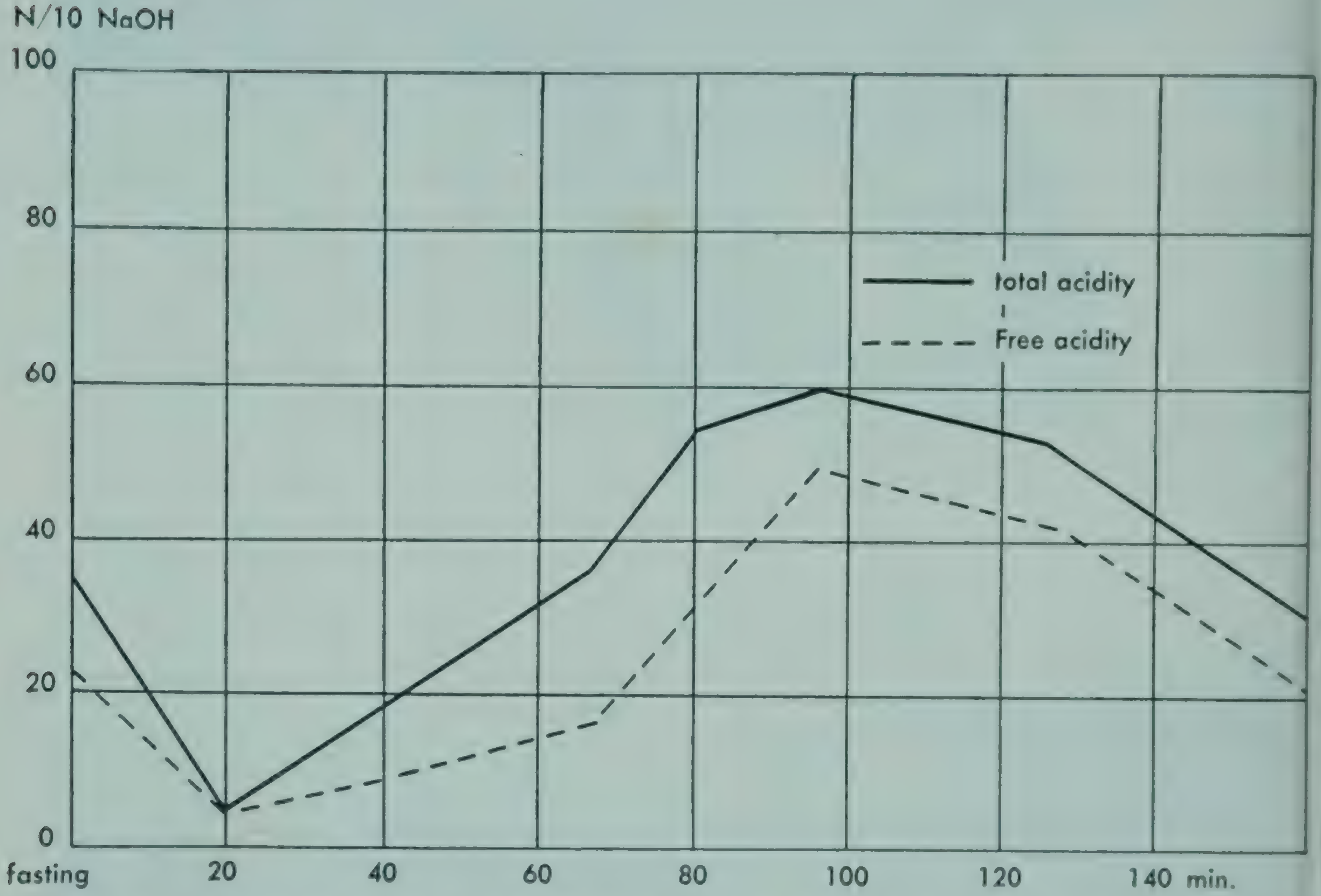

Fig. 2: Normal range of free and total acidity following test meal.

   b. An Ewald or other breakfast is administered. If alcohol is used; it can be given through the tube.
   c. Withdraw 5 cc. of gastric contents at fifteen-minute intervals until the acidity has returned to the same level as the fasting specimen.
   d. Examine the fasting specimen and each of the subsequent specimens for free hydrochloric acid, total acidity, and lactic acid.

The amounts of free hydrochloric acid and total acidity are expressed in degrees (the number of cubic centimeters of tenth-normal sodium hydroxide required to neutralize 100 cc. of the gastric contents using different indicators). The range of free hydrochloric acid is from 25 to 50 degrees. The range of total acidity is from 50 to 100 degrees.

ABNORMAL FINDINGS IN GASTRIC CONTENTS

Volume:

This is increased in chronic gastritis with deficient motor activity and in obstruction due to carcinoma or ulcer.

Mucus:

Mucus is increased in chronic gastritis.

Blood:

Blood or occult blood may be present in duodenal and gastric ulcers, carcinoma of the stomach, acute gastritis, and severe vomiting.

Bacteria and Yeast Cells:

Lactobacillus of Boas-Oppler, Sarcinae, yeasts, and miscellaneous bacteria are present when lactic acid and other organic acids are found in connection with pyloric obstruction and stagnation. The presence of large numbers of yeast cells indicates obstruction and fermentation.

Tubercle Bacilli:

These organisms may be swallowed in cases of pulmonary tuberculosis in which the sputum is negative. It is advisable in such cases to examine the gastric contents by culture and guinea-pig inoculation.

Enzymes:

Pepsin, rennin, and lipase are usually absent in pernicious anemia and combined lateral sclerosis. They are usually reduced in chronic gastritis and duodenitis.

Organic Acids:

Lactic acid and other organic acids are present with bacterial fermentation in cases of obstruction from carcinoma of the lower part of the stomach, obstructing ulcer, and chronic gastritis.

Free Hydrochloric Acid:

Hydrochloric acid is increased (hyperchlorhydria) in duodenal and gastric ulcers, chronic constipation, chronic cholecystitis, cholelithiasis, and excessive smoking.

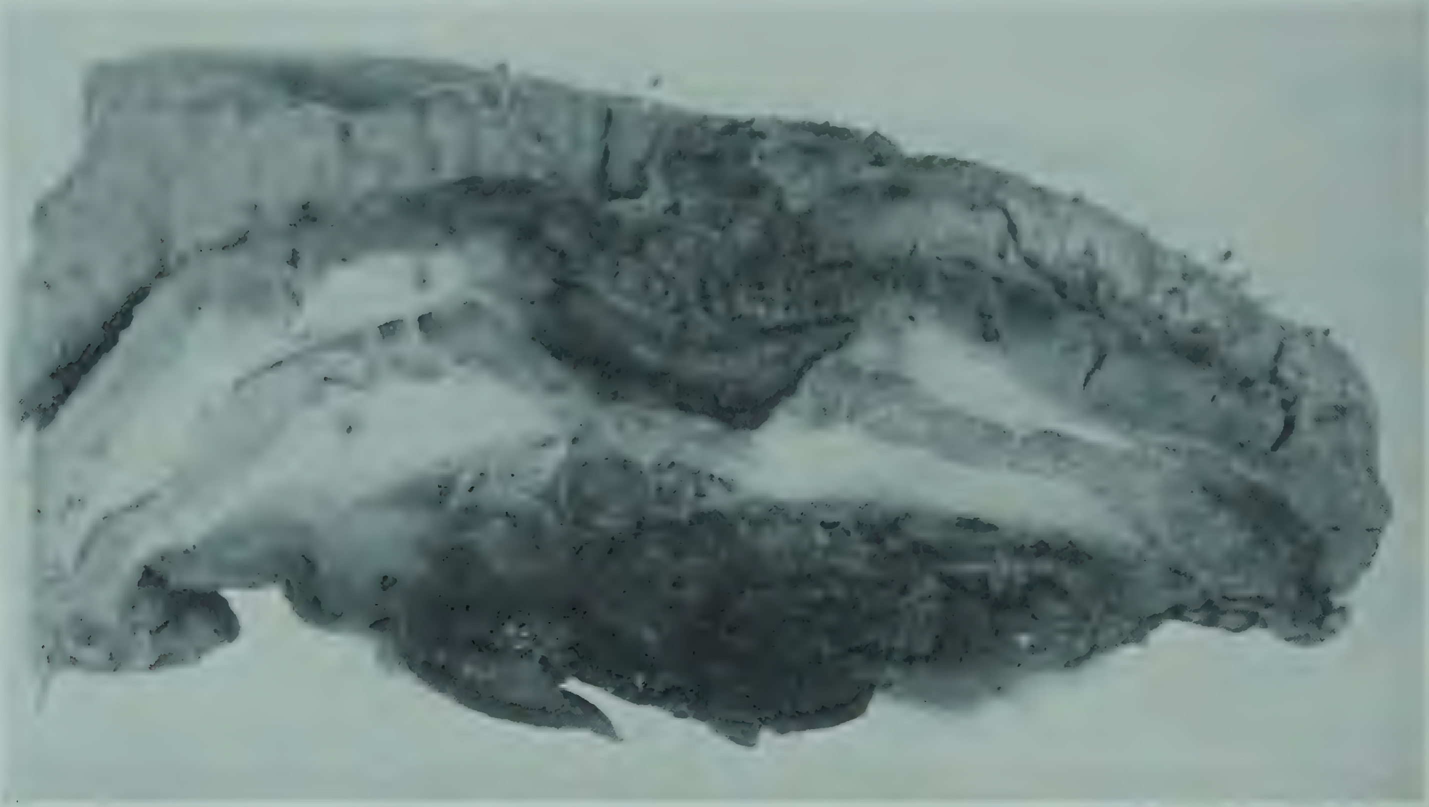

Fig. 3: Gastric ulcer, side view.

Hydrochloric acid is decreased (hypochlorhydria) in carcinoma of the stomach, gastritis and duodenitis, hyperthyroidism, gastric syphilis, visceroptosis, some cases of chronic appendicitis, secondary anemias, combined lateral sclerosis, some cases of pregnancy, chronic alcoholism, colitis, oral sepsis, and from 3 to 6 per cent of people over twenty years of age.

Hydrochloric acid may be absent (achlorhydria) in pernicious anemia, carcinoma of the stomach, gastric necrosis, idiopathic hypochromic anemia, septic conditions of the mouth, subacute combined degeneration of the cord, and about 4 per cent of people over fifty years of age.

Total Acidity: Total acidity parallels the free hydrochloric acid in those conditions causing hyperchlorhydria and hypochlorhydria.

Acid Deficit: When free hydrochloric acid is absent (achlorhydria), it is helpful to determine the acid deficit. This shows how far the acid secreted by the stomach falls short of saturating the protein and bases of the test meal. It represents how much hydrochloric acid must be added to the fluid before a test for free hydrochloric acid can be obtained. It can be estimated by titrating with tenth-normal hydrochloric acid, using dimethylaminoazobenzol as an indicator, until the fluid assumes a red color. The extent of the deficit is indicated by the number of cubic centimeters of the tenth-normal hydrochloric acid required for 100 cc. of gastric contents.

Food Particles:

Bits of food from previous meals indicate poor gastric motility or obstruction.

Leukocytes:

Leukocytes are rarely found in gastric contents after a test meal; however, they have been found in some cases of gastric carcinoma. Usually, they are partially digested and only the nuclei remain.

Tumor Cells:

Tumor cells may be found in suspected cases of carcinoma of the stomach (see p. 416). Preparation of smears with the Papanicolaou stain makes the cells identifiable for the trained cytologist.

DUODENAL CONTENTS

Examination of the duodenal contents will reveal certain information about the excretion of pancreatic ferments and excretion and infections of the biliary tract. Considering the effort and inconvenience to the patient for the information obtained, the value of the test is questionable.

Normal Duodenal Fluid

The normal, fasting, duodenal fluid is clear, viscid, and slightly alkaline. It may be colorless or light yellow owing to bile pigment.

The pancreatic ferments, amylopsin, lipase, and trypsin, are present. Urobilin is present in small amounts. Occasional leukocytes and epithelial cells are seen.

Normal values[2] for enzymes in duodenal contents, as determined by the methods indicated, are as follows:

Trypsin (Gross' method): from 25 to 50 cc. of 0.1 per cent casein solution digested by 1 cc. of duodenal fluid

Lipase (Myers and Fine's method): from 0.2 to 2.0 cc. of tenth-normal sodium hydroxide required to neutralize the acid formed

Amylase (Wohlgemuth's method): from 5 to 200 cc. of starch solution digested by 1 cc. of duodenal fluid

ABNORMAL DUODENAL FLUID

Pancreas:

The absence of amylopsin, lipase, and trypsin indicates deficient secretion or obstruction of the pancreatic duct.

Use of Secretin in Determining Pancreatic Function: Secretin has been used by some investigators to determine pancreatic function. Secretin is a hormone, which was discovered by Bayliss and Starling in 1902. When injected intravenously, it appears to wash out preformed enzymes from the pancreas instead of stimulating the formation of new enzymes.

Maddock and his colleagues[3] used secretin in determining the increase in pancreatic enzymes in the duodenal contents of a series of children. The procedures for determining the levels of trypsin, lipase, and amylase in this investigation are described in detail by Shwachman, Farber and Maddock.[4] Their results are shown in Table 1.

EFFECT OF SECRETIN ON TRYPTIC ACTIVITY: Normal infants and children and those with chronic nutritional disturbance reacted in about the same degree, with an appreciable increase in tryptic activity after the injection of secretin. There was a slight increase in tryptic activity in patients with pancreatic fibrosis, but the increase of actual units for one hour after the injection of secretin was so small that it was considered negligible. No explanation can be offered for the slight but definite decrease in tryptic activity in the patients with idiopathic celiac disease. Secretin had no effect on the zero value of the patients with selective trypsin deficiency.

TABLE 1: TOTAL ACTIVITY OF ENZYMES IN UNITS FOR ONE HOUR BEFORE AND ONE HOUR AFTER STIMULATION WITH SECRETIN

	No. of Cases	*Trypsin*		*Lipase*		*Amylase*	
		Before	*After*	*Before*	*After*	*Before*	*After*
Normal infants and children	5	4,325	6,720	1,573	2,682	3,293	5,780
		(55 per cent)		(70 per cent)		(75 per cent)	
Patients with pancreatic fibrosis	7	21.6	26.4	13.7	9.4	216.3	137.5
		(22 per cent)		(−31 per cent)		(−36 per cent)	
Patients with chronic nutritional disturbance	6	2,769	4,253	482	1,191	1,090	3,534
		(54 per cent)		(146 per cent)		(224 per cent)	
Patients with celiac disease	2	11,715	7,480	1,507	3,070	6,560	5,000
		(−36 per cent)		(104 per cent)		(−24 per cent)	
Patients with trypsin deficiency (two determinations)	1	0	0	397	1,147	1,610	3,200
				(189 per cent)		(99 per cent)	

EFFECT OF SECRETIN ON LIPOLYTIC ACTIVITY: A definite increase in lipolytic activity was observed in normal cases after the administration of secretin. The greatest increase occurred in the patient with selective trypsin deficiency. In chronic nutritional disturbance and idiopathic celiac disease, the increase was greater than in normal persons. There was a small but definite decrease in lipolytic activity after injection of secretin in patients with pancreatic fibrosis.

EFFECT OF SECRETIN ON AMYLOLYTIC ACTIVITY: Secretin produced a moderate increase in amylolytic activity in normal infants and children. There was a greater increase in the patient with selective trypsin deficiency and a much greater increase in patients with chronic nutritional disturbance. Small but definite decreases in amylolytic activity were observed in the patients with celiac disease and in those with pancreatic fibrosis.

Pratt[5] was able to study the function of the pancreas before and after the use of secretin in five adults with proved cases of cancer of the pancreas. Without the use of secretin, no evidence of pancreatic insufficiency would have been observed in four of the five cases. Pratt believes that it is proper to assume the presence of organic disease of the pancreas if low-enzyme values are found after the injection of secretin.

BILIARY SYSTEM

The presence of bilirubin indicates that the hepatic and common ducts are not completely obstructed. The absence of bile on one occasion, however, does not necessarily mean that the system is obstructed.

Increase of urobilin and the presence of urobilinogen are found in the hemolytic anemias or in blood destruction from other causes.

Microscopic examination that reveals a number of leukocytes would suggest infection or inflammation of the biliary tract or duodenum. Strongyloides stercoralis, Giardia lamblia, ova of Ascaris lumbricoides, and cysts or trophozoites of Endamoeba histolytica have been found on occasion. A few gram-positive cocci, most of which are dead, may be found, but bacteriological examination adds very little of clinical value.

BACTERIOLOGICAL EXAMINATION OF GASTRIC AND DUODENAL CONTENTS

This subject is discussed in Chapter 13 (p. 257).

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CHAPTER 6

Cerebrospinal Fluid

Information gained from laboratory examination of the cerebrospinal fluid makes it a worth-while procedure in the investigation of brain and spinal-cord tumors, infections of the meninges, and intrinsic and traumatic hemorrhage of the central nervous system. The serological examination of the cerebrospinal fluid is also helpful in investigating the presence or absence of syphilis of the central nervous system.

PHYSIOLOGY

It is believed that the cerebrospinal fluid is produced from the blood plasma by the choroid plexus in the ventricles of the brain. The fluid escapes from the fourth ventricle through the foramens of Luschka and of Magendie into the cisterna cerebellomedularis. About 80 per cent of the fluid flows up over the brain, and about 20 per cent flows downward around the spinal cord. The fluid that bathes the brain passes anteriorly beneath the medulla, pons, and mesencephalon. It passes between the mesencephalon and incisura of the tentorium and then spreads out around the brain in the subarachnoid space. In addition, the fluid accompanies the cerebral vessels into the brain substance via a sheath of pia-arachnoid surrounding each vessel. It is thus continuous with the Virchow-Robin space in the finer vascular branches. The remaining 20 per cent of the fluid flows downward around the spinal cord in its subarachnoid space, and from this there is a small return flow through the central canal of the cord.

To complete the circulation, most of the fluid is absorbed through the arachnoid villi. In addition, there is absorption through the capillaries in the sulci of the brain and through the blood and lymph vessels in the subarachnoid space of the cord. The amount of cerebrospinal fluid in the normal adult is from 120 to 150 cc.

The choroid plexus functions selectively concerning the materials that are allowed to pass on to the cerebrospinal fluid from the plasma. It permits alcohol, chlorides, sodium, magnesium, and carbon dioxide to pass through readily. Less permeability is noted for glucose, proteins, urea, chloroform, hexamethylenamine, and urethane. There is very little permeability for fibrinogen, bilirubin, cholesterol, complement, toxins, chemicals, and antibodies, natural or acquired. The permeability of the choroid plexus, however, appears to be increased in acute and chronic meningitis. Parr and Goodale[1] investigated 156 spinal fluids to determine the presence of isohemagglutinins representing the blood types. Of these, only twenty-one showed isohemagglutinins. The spinal fluids of this series represented a wide variety of illnesses, many of which did not primarily involve the central nervous system. A series of eighty spinal fluids investigated by Hermann and Halber[2] showed isohemagglutinins in only eleven specimens. It must be concluded that the occurrence of natural antibodies in the form of isohemagglutinins is limited and apparently capricious.

SPINAL PUNCTURE

A spinal puncture is usually carried out to secure fluid for diagnostic purposes or to drain off fluid to relieve pressure. The puncture is usually carried out in the lumbar region between the third and fourth, or between the fourth and fifth, lumbar vertebrae.

Procedure:

1. A lumbar puncture is usually carried out with the patient lying on the side and with the torso flexed to produce the greatest

separation of the vertebrae. The sitting position may be used, but the fluid pressure is not so accurate in this position.

2. The lumbar area is disinfected with an antiseptic. The operator should wear sterile gloves.

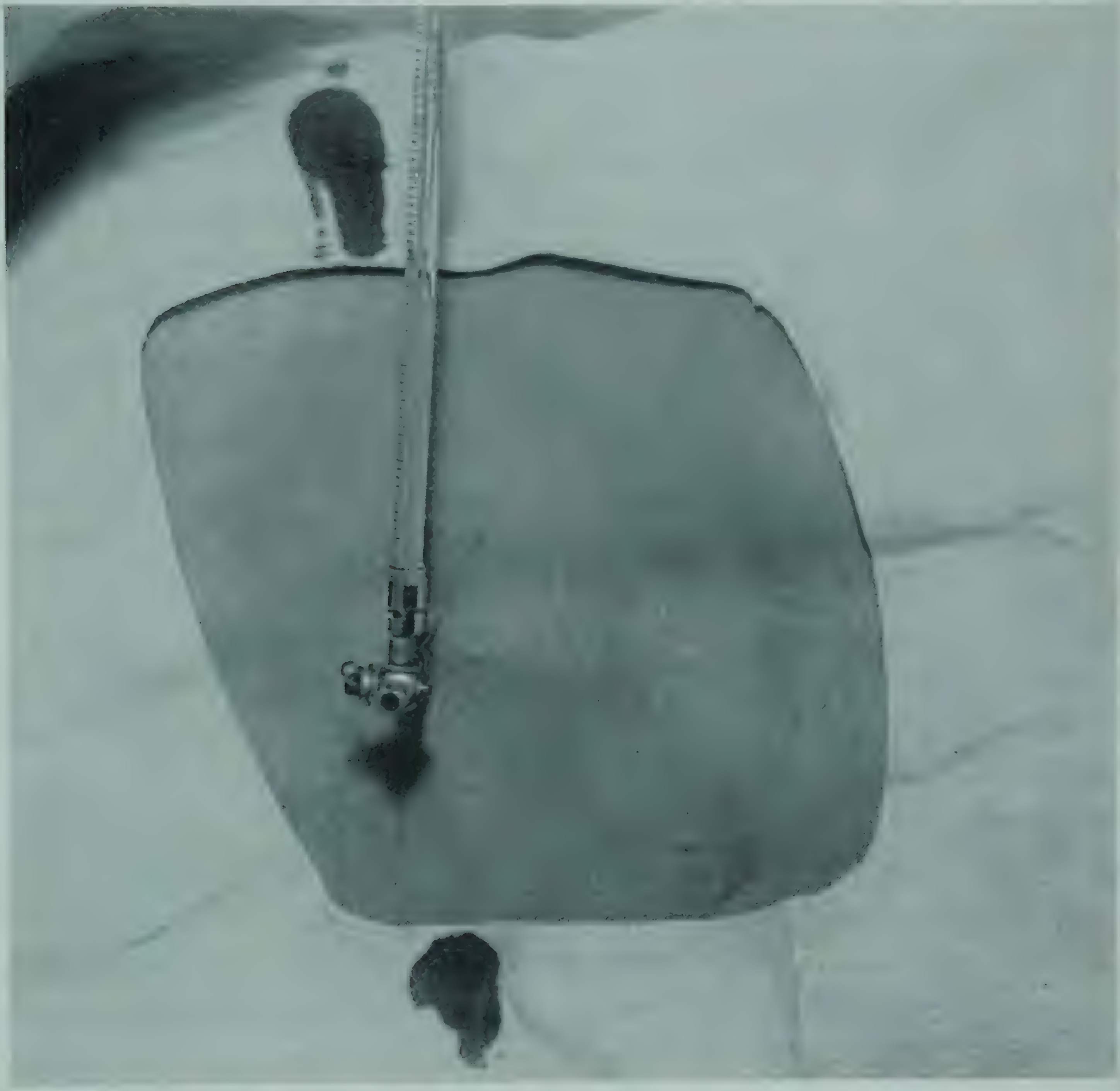


Fig. 1: Spinal puncture with needle and manometer in place.

3. A line from the crest of the ilium will usually indicate the appropriate site for the puncture. The space between the appropriate spinous processes can be identified by a palpable depression in the skin.
4. A small amount of 1 per cent procaine is injected at the appropriate site, and the needle is inserted slowly at right angles to both axes.
5. When a give is felt, the tip of the needle is in the subarachnoid space.

6. The stylet is removed, and clear fluid drips from the needle if it is in the proper place.
7. The pressure is taken before any fluid is drained off.
8. The fluid is collected in two tubes for laboratory examination. One tube may contain a small amount of potassium oxalate if the fluid tends to coagulate.
9. The stylet is then replaced, and the needle is removed. The needle hole is covered with a sterile dressing. The patient is kept in bed over night to minimize the chance of lumbar-puncture headache.

Sometimes a puncture of the cisterna cerebellomedularis is done when a block is present in the spinal cord. A ventricular puncture sometimes is used in infants and occasionally in adults when the neurosurgeon wishes to investigate the properties of the fluid in one of the lateral ventricles.

When the puncture is completed, the fluid should be examined immediately because the cell count and sugar values decrease on standing. In cases of meningitis, it is desirable to know the causative organism as soon as possible in order that appropriate therapy may be instituted. The desired tests should be listed in order of importance in case there is not enough fluid for all tests.

PHYSICAL CHARACTERISTICS

Pressure:

The normal pressure with the patient lying on the side is from 100 to 200 mm. of water or up to 8 mm. of mercury. When the puncture is done with the patient in the sitting position, the pressure is increased to almost twice that of the recumbent position. The pressure is also increased by crying, coughing, or struggling.

The compression of the jugular veins, or Queckenstedt's test,[3] is done routinely. This normally increases the intracranial pressure and spinal-fluid pressure; but when a subarachnoid block is present, the increased pressure does not register on the spinal-fluid monometer.

Cerebrospinal-fluid pressure is increased in certain brain tumors, hydrocephalus, meningitis, intracranial hemorrhage, brain abscess, cerebral thrombosis, decompensation, uremia, erythremia, edema of the brain, and sometimes anterior poliomyelitis and encephalitis.

The pressure is decreased in a block of the subarachnoid space of the spinal cord, after a recent lumbar puncture (particularly with headache), and in shock, fainting, dehydration, and obstructiv types of hydrocephalus.

Appearance:

The normal fluid is clear and colorless like water. The specifi gravity varies from 1.001 to 1.008. When blood is present in th first collecting tube, it becomes necessary to take specimens in second and third tube until the fluid is clear. If blood is presen in all tubes, it may be inferred that the blood is coming from central nervous-system hemorrhage and is not caused by the trauma of th procedure.

Color: A yellowish color of the fluid is called "xanthochromia." The color is usually due to altered hemoglobin from an intracrania hemorrhage. It may be due, however, to such pigments as bilirubi or carotin.

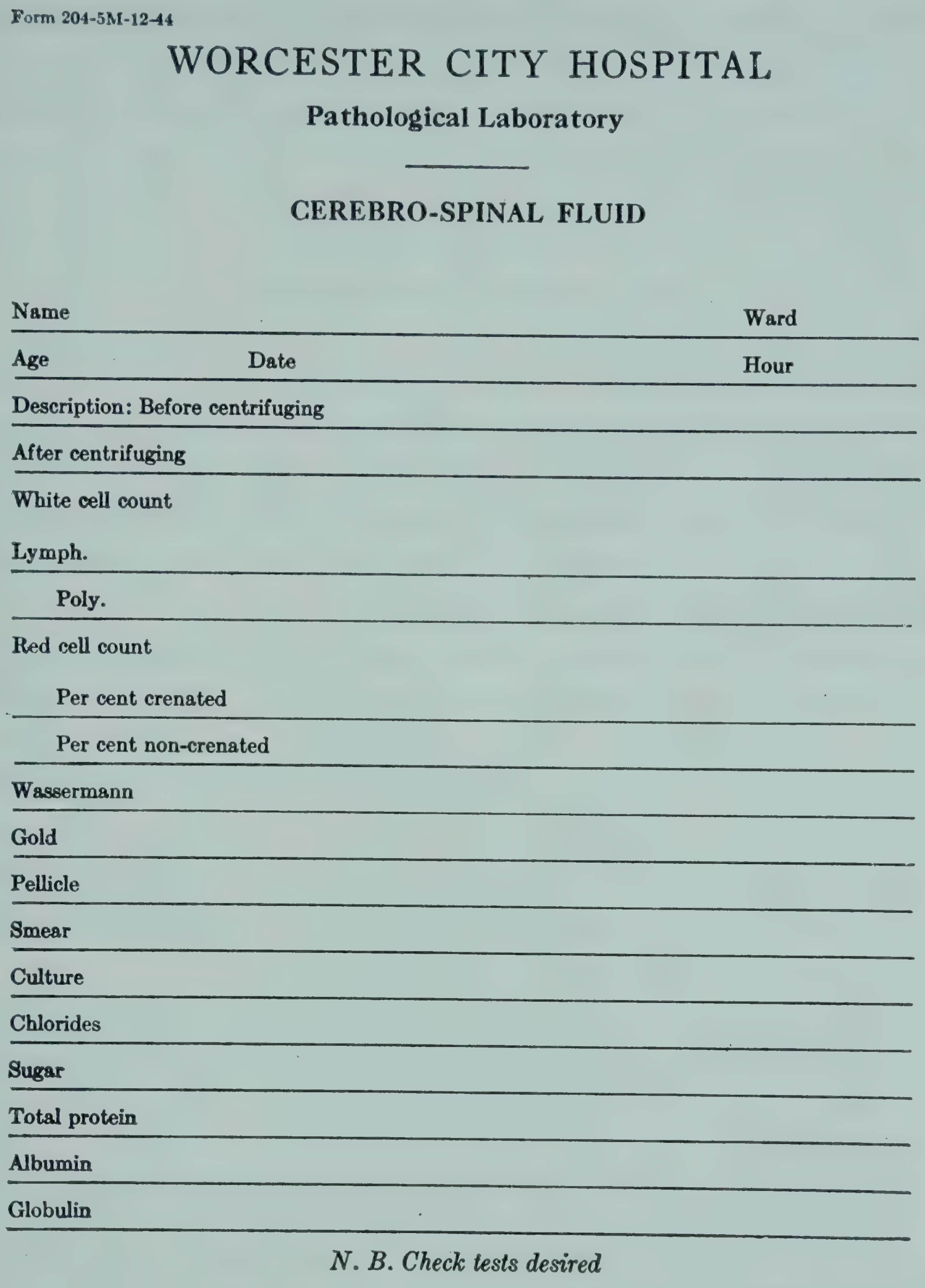
Turbidity: Cloudy fluids that have a ground-glass appearanc are seen in meningitis, which causes an increase in the cell count with the outpouring of large numbers of leukocytes.

Pellicle: If fibrinogen is present, a fine web may form when the spinal-fluid specimen is allowed to stand at room temperature. A fine web is most often seen in tuberculous meningitis, while particles of coagulated material sink to the bottom in purulent meningitis. A pellicle may also develop because of an intraspinal tumor, anterior poliomyelitis, general paresis, and occasionally epidemic encephalitis.

Cell Count:

The total cell count and differential count are valuable routine procedures. The normal cell count for persons over ten years of age is 0 to 10 lymphocytes. In infants, the normal range is 20 to 30 lymphocytes. No red cells are seen in the normal fluid, although the presence of a few may be the result of lumbar-puncture trauma. Fluids containing macroscopic blood from the lumbar puncture are not satisfactory for white-cell counts because the results will not be accurate. However, red-cell counts are done on bloody fluids as a basis for future comparison, that is continuation or cessation of hemorrhage.

Increased cell counts with polymorphonuclear leukocytes pre-



Form 204-5M-12-44

WORCESTER CITY HOSPITAL

Pathological Laboratory

CEREBRO-SPINAL FLUID

Name Ward

Age Date Hour

Description: Before centrifuging

After centrifuging

White cell count

Lymph.

Poly.

Red cell count

Per cent crenated

Per cent non-crenated

Wassermann

Gold

Pellicle

Smear

Culture

Chlorides

Sugar

Total protein

Albumin

Globulin

N. B. Check tests desired

Fig. 2: Cerebrospinal-fluid form.

dominating are found in all types of pyogenic meningitis, in brain abscess when it impinges upon the surface of the brain, and in early anterior poliomyelitis. Later in this disease lymphocytes predominate. The cell count is increased with lymphocytes predominating in tuberculosis meningitis, lymphocytic choriomeningitis, epidemic encephalitis, anterior poliomyelitis after a few days, some brain and spinal-cord tumors, syphilis of the central nervous system, and multiple sclerosis.

The cell count may or may not be increased in multiple sclerosis and herpes zoster.

CHEMICAL TESTS

Organic Compounds

Protein:

The normal total protein of cerebrospinal fluid from lumbar puncture is 15 to 40 mg. per 100 cc. The fluid obtained from cisterna-cerebellomedularis puncture is lower and ranges from 10 to 25 mg., while ventricular fluid frequently contains still less. The protein is made up chiefly of albumin (23 mg. per 100 cc.) with a small amount of globulin (5 mg. per 100 cc.).

Increase in Total Protein: In meningitis, the cells of the choroid plexus become more permeable and allow the passage of albumin, globulins (pseudoglobulin and euglobulin), and fibrinogen. The amount of protein parallels somewhat the extent of the inflammatory process in the meninges.

Although an increase in globulin is indicated roughly by Pandy's test, quantitative determination of the total protein is preferred.

Total protein is increased in the following:

1. Conditions due to infection: pyogenic meningitis, tuberculous meningitis, syphlitic meningitis, brain abscess that impinges on the meninges or ventricular wall, or tabes dorsalis (less so than the others)
2. Hemorrhage: cerebral, traumatic, or from brain tumors
3. Thrombosis: cerebral, spontaneous, or embolic
4. Virus disease: anterior poliomyelitis, encephalitis, or lymphocytic meningitis
5. Miscellaneous conditions: sterile meningitis from injecting chemicals, after convulsion, chronic alcoholism, or paralysis agitans

Glucose:

The glucose of the spinal fluid apparently comes from the glucose of the blood via the choroid plexus. Its level parallels that of glucose in the blood and normally ranges between 50 and 80 mg. per 100 cc. in adults and between 70 and 90 mg. per 100 cc. in children under ten years of age.

Glucose is increased (hyperglycorrhachia) in epidemic encephalitis and anterior poliomyelitis. It is also increased slightly in brain tumor and brain abscess when associated with intracranial pressure. If, however, the brain abscess communicates with the meninges, the glucose may not be increased because of the presence of glucose-splitting bacteria. Glucose is increased in certain systemic conditions, particularly diabetes mellitus and uremia. Determination of spinal-fluid glucose is helpful in investigating coma due to diabetes mellitus.

Glucose is decreased (hypoglycorrhachia) characteristically in purulent meningitis and tuberculous meningitis. This is owing to the splitting-up of glucose by the organisms concerned. There is slight decrease in glucose in meningovascular syphilis. Glucose is decreased in those fluids that have remained in the test tube for several hours. In view of this, it is important to determine the glucose level within two hours or to place the specimen in the refrigerator.

Miscellaneous Organic Compounds:

The determination of the remaining organic compounds in the cerebrospinal fluid is of little help to the clinician. These compounds with the conditions in which they are increased are listed in Table 1.

TABLE 1: COMPOUNDS AND CONDITIONS OF INCREASE

Compound	*Normal Range (per 100 Cc.)*	*Conditions Showing Increase*
Nonprotein nitrogen	12.5 to 30.0 mg.	Nephritis and uremia
Urea nitrogen	6.0 to 15.0 mg.	Nephritis and uremia
Urea	5.0 to 38.0 mg.	Nephritis and uremia
Residual nitrogen	2.0 to 6.0 mg.	Nephritis and uremia
Uric acid	0.4 to 2.8 mg.	Nephritis, uremia, and meningitis
Lactic acid	8.0 to 27.0 mg.	Meningitis

INORGANIC COMPOUNDS

Chlorides:

The normal range of chlorides as sodium chloride is from 720 to 750 mg. per 100 cc. for adults. Infants have somewhat lower values, namely from 650 to 720 mg. per 100 cc. The chlorides are decreased particularly in tuberculous meningitis from 450 to 600 mg. per 100 cc. and in pyogenic meningitis from 640 to 700 mg. per 100 cc. An insignificant decrease may be noted in epidemic encephalitis, anterior poliomyelitis, central-nervous-system syphilis, brain tumors, and lymphocytic meningitis.

Miscellaneous Inorganic Constituents:

The inorganic compounds listed in Table 2 are not determined because they do not help in clinical diagnosis.

TABLE 2: MISCELLANEOUS INORGANIC COMPOUNDS

Constituent	*Normal Range (per 100 Cc.)*	*Conditions Showing Increase*	*Conditions Showing Decrease*
Calcium (ionized)	4.5 to 5.50 mg.	Pyogenic and tuberculous meningitis, hydrocephalus, brain tumors	Uremia
Phosphate	1.5 to 2.00 mg.	Uremia, pyogenic meningitis, tuberculous meningitis, hydrocephalus, brain tumors	
Cholesterol	0 to 0.22 mg.	Pyogenic meningitis, brain tumor, cerebral hemorrhage	

The following inorganic constituents are also present in the cerebrospinal fluid: sodium (300 to 340 mg. per 100 cc.), potassium (11 to 17 mg. per 100 cc.), and magnesium (1.0 to 3.6 mg. per 100 cc.).

PRECIPITATION TESTS

COLLOIDAL-GOLD REACTION

The principle of this test is based upon the fact that in certain pathological cerebrospinal fluids sodium chloride precipitates colloidal gold with characteristic color changes in standard dilutions. Normal fluids prevent this precipitation, and the color does not change from the original deep red. The reaction is thought to be due to increased amounts of globulin in the pathological fluids. It is of the utmost importance that no blood be present in the fluid because it will cause false positive reactions.

Procedure:

The test, consists of making dilutions of cerebrospinal fluid with 0.4 per cent sodium-chloride solution, beginning with a 1:10 dilution in the first tube and ending in the tenth tube with a dilution of 1:5120. Five cc. of the deep, red-gold chloride reagent are added to each tube, and the tubes are allowed to stand overnight at room temperature.

Interpretation:

In reading the results, the tubes are divided roughly into three zones from left to right (Zones I, II, and III). In order to plot a graph, the various color changes are given numbers as follows:

Deep red	0
Red-blue	1
Violet-purple	2
Blue	3
Pale blue	4
Clear and decolorized	5

Normal fluids may read “0000000000” or “1110000000.” If a fluid shows readings of “2221000000” or “0002220000,” the test should be repeated and checked against the serological test and the cell count.

A Zone-I reaction, showing maximum precipitation (decolorized) in the first two to five tubes, is suggestive of general paresis (paretic curve), taboparesis, and multiple sclerosis. This curve is read “555-5432000.” The Zone-II reaction (1123210000) is called the “tabetic,” or “luetic,” curve, because it is seen in tabes dorsalis and meningovascular syphilis. The Zone-III reaction (0001234530) is seen in purulent meningitis, tuberculous meningitis, and aseptic meningitis. This type of curve may also occur in anterior poliomyelitis and epidemic encephalitis, but unfortunately it is not diagnostic of either disease.

MASTIC TEST

The gum-mastic reagent for this test is easier to prepare than the gold chloride, but the test is less sensitive than the colloidal-gold test. Complete precipitation of the mastic corresponds to complete decolorization of the colloidal-gold solution.

Procedure:

In the first of five tubes, 1 cc. of a 1:4 dilution of spinal fluid is placed. (The diluting fluid is an alkaline-saline solution.) The dilutions are carried through to 1:64 in the fifth tube. A sixth tube, with alkaline-saline solution, is used as a control. Next, add 1 cc. of mastic solution to each tube, and allow the tubes to stand at room temperature overnight. Curves may develop that parallel those of the colloidal-gold test.

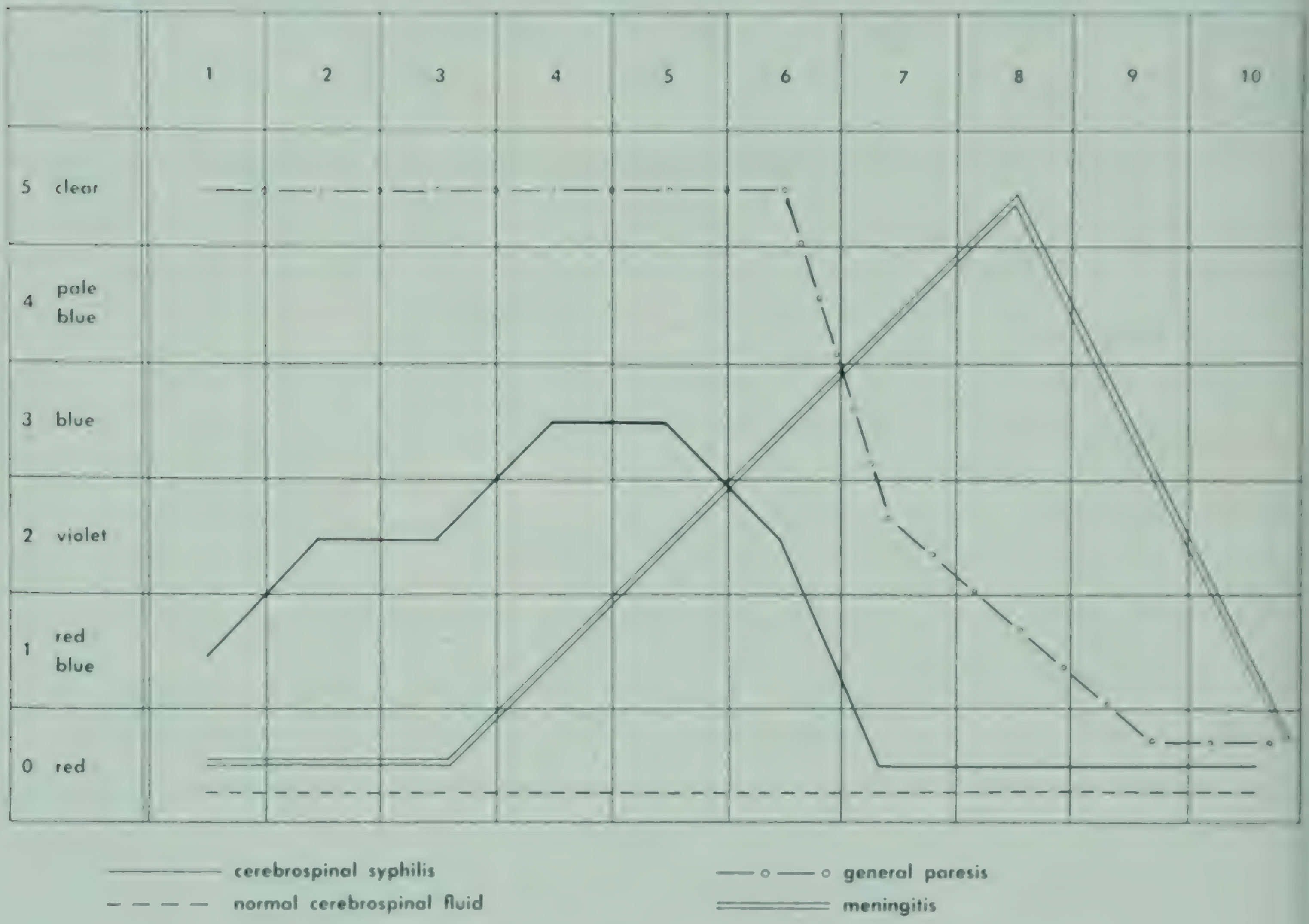


Fig. 3: Reactions in colloidal-gold test.

Interpretation: The test is read according to the amount of precipitation as follows:

Precipitation

None	0
Slight	1
Moderate	2
Complete	3

Three curves are seen corresponding to those of the colloidal gold: Zone I (paretic curve), Zone II (tabetic, or luetic curve), and Zone III (meningitic curve).

EXAMINATION FOR SYPHILIS

In examining the cerebrospinal fluid for syphilis (see p. 499), a complement-fixation test, such as the Wassermann test, is preferred. The Kahn test, however, has been used extensively for this purpose. The cerebrospinal fluid may give positive tests in both congenital and acquired syphilis, even though the blood tests are negative at the time. The test is positive in about 85 per cent of patients with meningovascular syphilis, 70 per cent of patients with tabes dorsalis, and almost 100 per cent of patients with general paresis. Neither complement-fixation tests nor flocculation tests indicate the type of syphilis. The colloidal-gold or colloidal-mastic tests, therefore, should be included to give a clue to the type of syphilis.

MISCELLANEOUS TESTS

LEVINSON TEST

This test, as described by Levinson,[4] is helpful in the diagnosis of tuberculous meningitis (see p. 498). It is based upon the fact that the ratio between the alkaloidal precipitate formed by sulfosalicylic acid and the metallic precipitate formed by mercuric chloride may be changed from normal in tuberculous meningitis.

Procedure:

1. Pipet 1 cc. of cerebrospinal fluid into each of two small test tubes.
2. Add 1 cc. of a 1 per cent solution of mercuric chloride to the fluid in one tube.
3. Add 1 cc. of a 3 per cent solution of sulfosalicylic acid to the fluid in the second tube.
4. Shake the tubes and stopper them. Leave them at room temperature for twenty-four hours.
5. Measure the height in millimeters of the precipitate in each tube.

Interpretation:

In tuberculous meningitis, the precipitate in the mercuric-chloride tube is three times as great as that in the sulfosalicylic-acid tube. In pyogenic meningitis, a reverse ratio usually results, that is the sulfosalicylic-acid precipitate may be two or three times as great as that in the mercuric-chloride tube.

SERUM TEST FOR MENINGOCOCCUS MENINGITIS

Procedure:

1. Add a few drops of antimeningococcus serum to 1 cc. of cerebrospinal fluid.
2. Place the tube and a control tube containing only cerebrospinal fluid in an incubator at 125.6° F. (52° C.) for a few hours.

Interpretation:

A positive reaction is characterized by the appearance of a white cloud. This is indicative of meningococcus meningitis, but it may occur in other forms of meningitis. Both tubes may develop a cloud while in the incubator.

TRYPTOPHAN TEST

This is a test for tryptophan in the spinal fluid, which is recommended by Lichtenberg.[5]

Procedure:

1. Add 15 to 18 cc. of concentrated hydrochloric acid and 2 or 3 drops of 2 per cent formaldehyde solution to a test tube containing 2 or 3 cc. of cerebrospinal fluid. Shake, and allow to stand for five minutes.
2. Overlay this mixture with from 1 to 2 cc. of a 0.06 per cent solution of sodium nitrite, and allow it to stand two or three minutes.

Interpretation:

In tuberculous meningitis, a violet ring forms at the junction of the two layers. A negative reaction has a brown ring, or there is complete absence of color.

BACTERIOLOGICAL EXAMINATION

The bacteriological examination of the cerebrospinal fluid is discussed in Chapter 13 (p. 258).

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CHAPTER 7

Sputum

ORIGIN

Sputum is an exudate, which is formed in the course of a pulmonary, bronchial, or tracheal infection. The material accumulates in the bronchi and is gradually carried up the trachea by the upward sweep of the cilia of the mucosa. After coughing, the sputum is forced from the upper trachea to the mouth. Sputum does not include saliva or secretions from the nose, but it is difficult to prevent the mixture of sputum and saliva.

Sputum may also be produced from the fluid of pulmonary edema, or from the rupture of a cavity or abscess into a bronchus. Rupture of a vessel may result in blood-tinged sputum or a specimen of almost pure blood.

COLLECTION

The patient should be instructed to save for examination only that material that is coughed up from the bronchi. Mucus from the posterior nasopharynx and saliva are not satisfactory for sputum examination. Sputum should be collected in a sputum cup for routine examination and in a sterile, wide-mouthed jar with a sterile cover for culture. The patient should be advised against contaminating the outside of the container because this is a hazard for the nurses and technicians who must handle it.

Morning specimens are desirable because the patient usually has a coughing spell after waking up, clearing the bronchi and trachea of material that has accumulated during the night. Sometimes, a twenty-four-hour or a seventy-two-hour specimen is requested for a concentration test for mycobacteria tuberculosis.

In some cases of pulmonary tuberculosis, there is no sputum, possibly because the patient swallows it. This is especially true of children and infants. In such cases, a gastric lavage should be done to rule out pulmonary tuberculosis by inoculating the stomach contents into a guinea pig.

PHYSICAL CHARACTERISTICS

Volume:

Although it is not necessary to measure accurately the volume of sputum per day, increases and decreases should be noted. The amount is roughly noted by the number of sputum cups filled in twenty-four hours. Large amounts, up to a liter, are produced in tuberculosis with cavitation, lung abscess, edema of the lungs, and bronchiectasis. Progressive increase in the amounts of sputum in the above diseases indicates that they are becoming worse, and conversely decrease in the amounts of sputum indicates that they are improving.

Consistency:

A mucoid sputum is found in asthma and bronchitis; a purulent or mucopurulent sputum is found in rupture of an abscess or of empyema into a bronchus, in pulmonary tuberculosis with cavitation, and in bronchiectasis; a serous sputum is found in edema of the lung. In lobar pneumonia, the sputum is tenacious and rust-colored.

Some sputums form three layers when placed in a cylinder. Such layer formation may be seen in pulmonary abscess, gangrene, and bronchiectasis.

Color:

The color of sputum frequently suggests the nature of the underlying process. It is colorless and translucent when it is made up of mucus only. When large numbers of pus cells are present, it ranges from white to yellow. A greenish color is imparted by bile pigment in the presence of jaundice, for example in pneumonia with jaundice. Bacillus pyocyaneus gives it a green color. A gray to black color is imparted by the expectoration of anthracotic particles and is seen particularly in stokers, miners, and chimney cleaners. A ruptured amebic abscess gives the sputum an anchovy-sauce appearance. The sputum of pneumonia and pulmonary infarct has a rusty color, while that of heart failure is brown. Blood-streaked sputum is produced at times in pulmonary tuberculosis and bronchiectasis. The inhalation of flour by bakers and flour handlers results in a white sputum. Pure blood with bits of mucus is seen in hemoptysis.

Odor:

Sputum from tuberculous cavities has a sweet odor. A foul odor is found in lung abscess, gangrene of the lung, and necrotizing bronchogenic carcinoma late in the disease.

Casts of Bronchi:

If a diphtheritic membrane extends from the trachea into the bronchi, it forms a cast of these structures. Fragments of these casts may be found in the sputum. Tiny, nonbranching casts are sometimes produced in lobar pneumonia.

Dittrich's Plugs:

These are pinhead-sized, yellow or grayish bits of foul-smelling, caseous material, which are coughed up in chronic bronchitis and bronchiectasis. The cheese-like material from tonsil crypts is sometimes included in this category. When crushed and examined under the microscope, they are made up of granular debris, bacteria, fat globules, and fatty-acid crystals.

Pneumoliths:

These are made up chiefly of calcified fragments of tuberculous tissue. Foreign bodies that have been covered with calcium may also be coughed up after eroding into a bronchus.

MICROSCOPIC EXAMINATION

UNSTAINED SPUTUM

Both the unstained and stained sputums reveal information that is helpful in diagnosis. It is worth the time taken to examine the sputum carefully and to pick out suspicious particles of caseous material for microscopic examination.

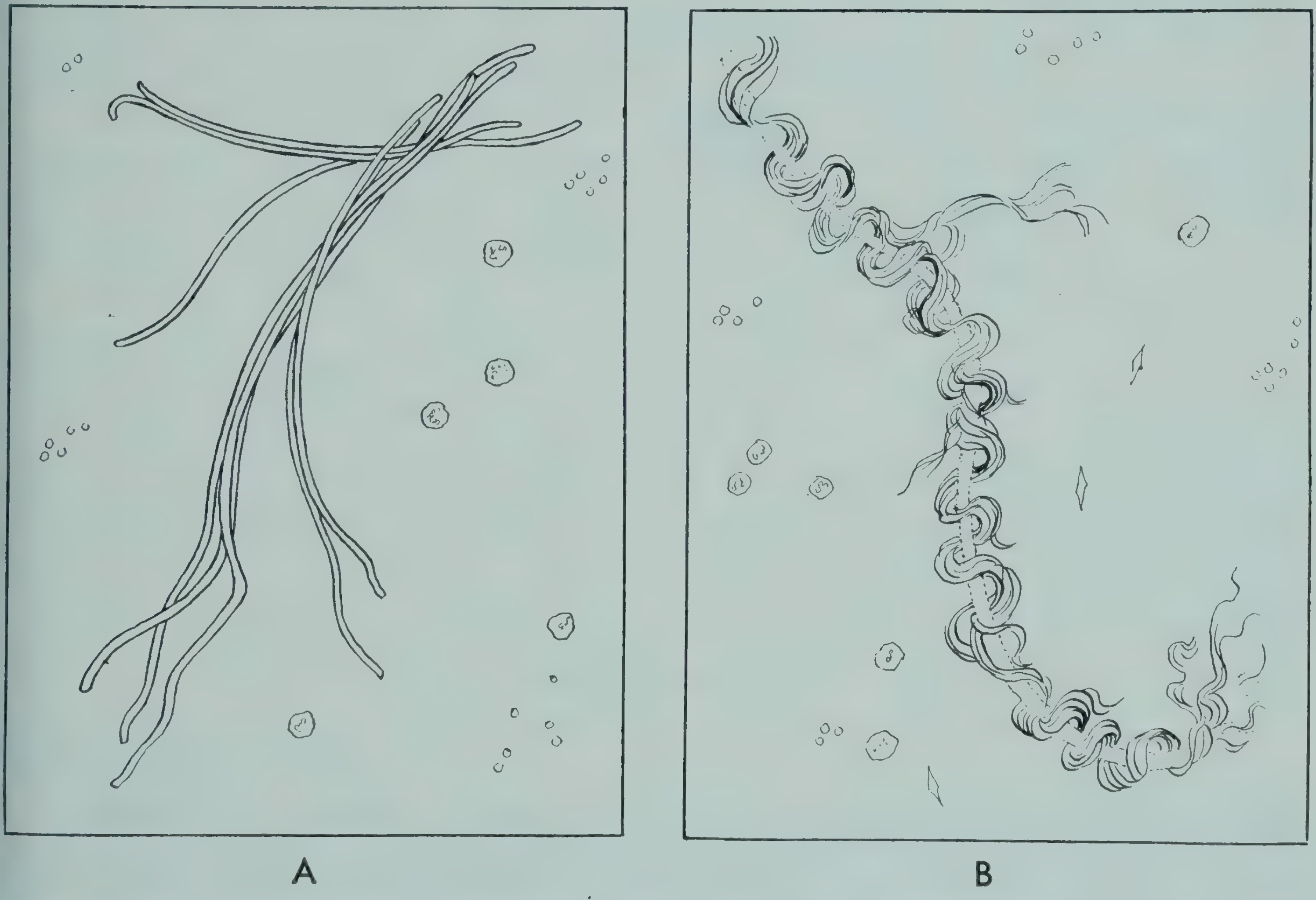

Fig. 1: *A*: Elastic fibrils. *B*: Curschmann's spirals.

Curschmann's Spirals:

These are tiny, white or yellow, wavy threads, sometimes coiled, which are found characteristically in asthma, particularly after an attack. They are sometimes found in chronic bronchitis. The microscopic picture suggests a coiled mass of mucus with enmeshed leukocytes.

Elastic Fibers:

Elastic tissue is normally present in the walls of bronchioles and alveoli, and the presence of its fibers in the sputum indicates destruction of the walls. These elastic fibers are wavy, highly refractive, curled, and split at the ends. They may be single or in bundles and sometimes have an alveolar arrangement. They are found in pulmonary abscess, gangrene, and tuberculosis. They are sometimes seen in early tuberculosis before the mycobacteria tuberculosis are found; and in advanced tuberculosis, there are frequently large numbers.

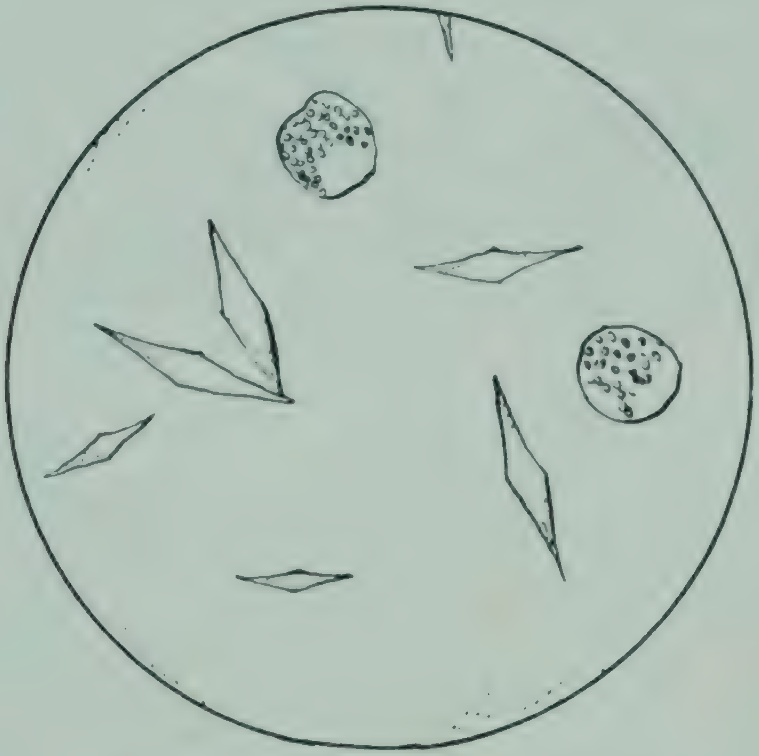

Fig. 2: Charcot-Leyden crystals.

Cotton fibrils, elastic fibers from food, and Leptotrichia buccalis may be confused with elastic fibers from the lungs.

Crystals:

The most important crystals that occur in the sputum are Charcot-Leyden crystals. They occur chiefly in bronchial asthma and are usually not found until the sputum has stood for some time. They are also found in the feces with animal parasites, and in the clotted blood of leukemia. These crystals are pointed, colorless, needle-like structures.

Other crystals that may be found in sputum are cholesterol, hematoidin, and fatty-acid crystals, which are found in bronchiectasis and pulmonary abscess. Fatty-acid crystals are also found in Dittrich's plugs.

Pigmented Cells:

The most important pigmented cells are the so-called "heart-failure" cells. These are large mononuclear cells, which are filled with yellow to brown hemosiderin. They are found in chronic passive congestion of the lungs resulting from decompensation.

Carbon-containing cells are found in the sputum of persons with marked anthracosis. Similar cells with smaller, gray-black particles are seen in the morning sputum of those who live in a smoky atmosphere or who inhale tobacco smoke extensively.

Myelin Globules:

These globules occur in the sputum of persons who appear to be normal. They have no diagnostic significance and are mentioned because they may be confused with blastomycetes. Myelin globules are colorless, round, oval, or pear-shaped bodies, suggestive of fat droplets. Some may have concentric or spiral markings, and they may occur free or within large mononuclear cells.

Animal Parasites:

Although animal parasites are rare in the sputum, a few have been reported. Infection with the lung fluke, Paragonimus westermani, produces what is known as "oriental hemoptysis." It occurs in the Orient and South America; in North America, it occurs only in those persons who have contracted the disease elsewhere and have migrated to this continent. The parasite is ingested with raw crabs and crayfish. In the duodenum, the metacercariae leave their cysts, go through the intestinal wall, and then traverse the peritoneal cavity to the diaphragm. They pass through the diaphragm into the lungs, where they lodge near bronchioles. Here they develop into the adult form and are walled-off by the host. They cause the production of blood-tinged sputum, which contains the ova.

An hydatid cyst occurs rarely in the lungs; and if the cyst breaks down, hooklets may be found in the sputum.

Endamoeba histolytica is usually found in the feces of infected persons. The disease is endemic and universal because it is transmitted from man to man without going through an intermediate host. In some cases of amebiasis, the parasites form an abscess in the liver, which may rupture through the diaphragm into the lungs. When this occurs, trophozoites or cysts may be found in the sputum.

Larvae of Strongyloides stercoralis and of the hookworm have been found in the sputum. A protozoon, not unlike Trichomonas hominis, has been found in the sputum of a patient with gangrene of the lung. It is apparently, however, an incidental finding and not the causative agent.

STAINED SPUTUM

Eosinophils:

These cells are easily demonstrated by staining the sputum with Wright's stain. They are present in large numbers in bronchial asthma and are practically diagnostic of this condition. They occur in smaller numbers in cardiac asthma due to left ventricular failure from aortic stenosis, aortic insufficiency, or hypertension.

Leukocytes:

These cells are readily recognized after using Gram's stain. They occur in all sputums from pyogenic infections of the lungs and tuberculosis with cavitation.

Tumor Cells:

By staining a cell block from sputum with hematoxylin and eosin, or by staining a smear with the Papanicolaou technic,[1] it is possible to identify tumor cells. Tumor cells, however, will be found more frequently if material is obtained directly from the bronchi during bronchoscopy. This material, properly fixed and stained by the Papanicolaou technic, will give a much higher proportion of positive findings than a similar number of sputum examinations using material obtained by other methods.

BACTERIOLOGICAL EXAMINATION

Bacteriological examination of the sputum is discussed in Chapter 13 (p. 260).

FUNGUS EXAMINATION

Fungus examination of the sputum is discussed in Chapter 15 (p. 292).

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CHAPTER 8

Feces

INTRODUCTION

The intestinal contents in the cecum and ascending colon are liquid As they pass through the colon, fluid is absorbed, leaving the normal formed feces. The constituents of normal feces are food residues, secretions from the intestinal wall, bile, a few leukocytes, degenerated epithelial cells, and bacteria. Food residues, with the exception of cellulose when present, constitute a very small portion of the stool. In the early stages of starvation, the nature and constituents of the feces continue to be the same, although later the volume is reduced. A stool is passed normally once or twice in twenty-four hours, but variations from this routine down to one stool in three days are experienced by some persons.

A thorough examination of the feces yields information that cannot be gained otherwise. In fact, the clinician can glean much information by merely inspecting the feces before sending it to the laboratory. Inspection reveals the form, consistency, odor, color, and presence or absence of blood, mucus, and visible parasites. Chemical tests, microscopic examination, and bacteriological examination must be done in the laboratory.

MACROSCOPIC EXAMINATION

Form:

The normal stool is soft, molded in the form of a cylinder, and measures 2.5 cm. (1 inch) in diameter in adults. It may be ribbon-like or of smaller diameter in carcinoma of the sigmoid or rectum, or in lymphopathia venereum of the rectum.

Consistency:

The stool is normally soft but maintains its cylindrical shape. Hard, dry stools are seen in constipation; in fact, some constipated persons pass so-called "scybalous stools," made up of round, dry masses. Mushy to liquid stools are passed following catharsis and during diarrhea. In diarrhea caused by Endamoeba histolytica or bacillary dysentery, the stools are small and frequent, containing mucus and blood with practically no fecal matter.

Quantity:

The quantity of stool passed daily varies slightly with the food intake and with the type of food, for example the quantity is greater when the person is on a vegetable diet. The normal person passes from 100 to 200 gm. (3 to 6½ ounces) in twenty-four hours.

Odor:

The odor of feces is normally due to the presence of indole and skatole and, in some instances, to hydrogen sulfide, methane, and phosphine. The strength of the odor depends upon the amount of meat ingested and upon the activity of bacteria. The odor is less with a vegetable or milk diet. A sour odor due to fatty acids is noted in the normal and mild diarrheal stools of infants. With severe diarrheas of children and adults, there may be a putrid odor. A very foul odor is present in syphilitic and carcinomatous ulceration of the rectum. A spermlike odor, due to cadaverine, is noted in the stools of cholera.

Color:

The normal color is from light to dark brown, but it may vary because of food or medication; for example spinach tends to make a stool green, and chocolate eaten in large quantities makes it gray. Medication in the form of iron salts makes a stool black.

In obstructive jaundice, stools are putty or clay-colored because of absence of bile pigment or an excess of fat. Putty-colored stools are also seen in pancreatic disease and tuberculous peritonitis because of the presence of fat. When a large amount of pus is present, they are grayish; and in cholera, they are almost colorless. In infantile diarrhea, they are green because of the presence of biliverdin. Black, or so-called "tarry," stools are seen in hemorrhage high up in the intestinal tract, for example ruptured esophageal varices and peptic ulcer. Stools streaked with red blood indicate that the source of the hemorrhage is low in the intestinal tract, that is carcinoma of the rectum or internal or external hemorrhoids. A stool the color and consistency of anchovy sauce is characteristic of amebic dysentery.

Mucus:

The normal stool contains very little or no mucus. In minor inflammations, as in infected diverticula, small amounts may be seen. In amebic and bacillary dysentery, there is much mucus mixed with fluid and blood. In mucous colitis, strings of mucus are passed that simulate tapeworms, including what appear to be segments. Mucus is also passed by persons with intussusception.

Concretions:

Gallstones are sometimes passed from the gallbladder into the duodenum and appear in the stool. Concretions originating in the intestine (enteroliths) are rare. Sandlike grains usually prove to be seeds. Foreign bodies are sometimes ingested and passed in the stool by children and psychotic patients.

Curds:

Imperfect digestion of fat or casein results in the formation of white, curdlike masses in the stools of infants. If made up of fat, the Sudan-III test will be positive; and if composed of casein, the stools will become tough after immersion in 10 per cent formaldehyde for twenty-four hours.

CHEMICAL EXAMINATION

Occult Blood:

In bleeding lesions of the upper intestinal tract, gross blood is not detected macroscopically in the stool because of the effect of digestion. In such cases, it becomes necessary to do a chemical test for occult blood. This is carried out by either the guaiac or the benzidine tests, neither of which is a test for iron. It is important to rule out the bleeding from the gums and a diet rich in meat as causes of a positive, occult-blood test.

The occult-blood test may be positive from ruptured varicose veins of the esophagus or stomach; swallowed blood from pulmonary hemorrhage; rupture of aortic aneurysm into the esophagus; carcinoma of the esophagus, stomach, or intestine; ulcer of stomach or duodenum; intestinal ulcers due to typhoid fever or tuberculosis; embolism of the superior mesenteric artery or one of its branches; venous thrombosis of mesenteric veins; ulcerative colitis; bacillary and amebic dysentery; volvulus; intussusception; cicatrizing enteritis; purpura; hemophilia; hemolytic jaundice; and hemorrhoids.

Bile Pigments:

The brown color of normal feces is due to urobilin. Bilirubin upon entering the intestine undergoes reduction to urobilinogen by the action of bacteria, and urobilinogen is changed to urobilin (stercobilin) by oxidation. Bilirubin is not found in the normal stool, but small amounts may be found in diarrhea and obstructive jaundice.

In obstructive jaundice in which the obstruction is complete, there is no urobilin to color the stool; consequently, it has a putty or clay color. After removal of the obstruction, the brown color returns to the stool. By testing the stool with saturated, mercuric-chloride solution (Schmidt's test) or Ehrlich's aldehyde reagent, the return of urobilin is indicated by the development of an appropriate color change.

Sometimes the quantitative determination of urobilin is of value. The normal range, according to the method of Watson,[1] is from 40 to 280 mg. in twenty-four hours, but the majority fall between 100 and 250 mg. Partial or complete obstruction of bile outflow reduces the urobilin markedly (5 mg. or less in twenty-four hours)[2]; but in jaundice from hepatitis, there is only moderate reduction. The output of urobilin is increased in red-blood-cell destruction, as in hemolytic jaundice and pernicious anemia; and in secondary anemia from loss of blood, it is normal or slightly decreased.

Total Fat:

On an ordinary diet, the total fat ranges from 7.3 to 27.6 per cent of the dry stool in twenty-four hours. The total fat is composed of neutral fat, fatty acids, and combined fatty acids, or soaps. Total fat is increased in the feces (steatorrhea) in ileitis, extrahepatic obstructive jaundice, pancreatitis, obstruction of the pancreatic duct, sprue, and celiac disease.

MICROSCOPIC EXAMINATION

Food Remnants:

Remnants of food are seen in normal stools, and they must be recognized as such. Fibers, cells, and hairs (down) from vegetables and fruit are seen in normal stools. Particles of starch and meat fibers are seen in the stools of diarrhea. The starch particles may be identified by the blue color with Lugol's solution, while meat fibers are recognized by the cross striations. Connective-tissue and elastic fibers may also be found in the course of a microscopic examination. All of the above-mentioned remnants are found when there has been excessive ingestion or deficient digestion.

Neutral fats, fatty acids, and soaps are also found occasionally in the stool. Neutral fat can be identified easily by the Sudan-III stain and examination under the microscope. Fatty-acid crystals appear like groups of phonograph needles; and soaps appear as well-defined, yellow, amorphous flakes or rounded masses. Small amounts of neutral fat and fatty-acid crystals may be found in the normal stool. An increase in neutral fats suggests pancreatic deficiency due to acute and chronic pancreatitis, carcinoma of the head of the pancreas, or stone in the pancreatic duct. An increase in fatty acids suggests deficient absorption due to lack of bile, as in obstructive jaundice.

Cells:

A few, partially degenerated, epithelial cells are seen in the normal stool. Large numbers are seen in inflammatory processes of the colon. Leukocytes are found in large numbers in bacillary dysentery and ulcerative colitis. In amebic dysentery, only a few leukocytes are found together with a few red cells. Macrophages, in addition to leukocytes are found in bacillary dysentery. Eosinophiles are found in the mucus in allergic conditions of the intestine.

Erythrocytes are seen only when the bleeding is in the colon or anus. The formation of clumps of three or more erythrocytes is said to be characteristic of amebic dysentery.

Crystals:

Various crystals are found in the stool as follows:

Fatty-acid: in deficient absorption due to lack of bile, as in obstructive jaundice
Calcium-oxalate: after eating certain vegetables
Charcot-Leyden: in ulcerative conditions of the intestine
Hematoidin: after hemorrhage into the intestine
Bismuth-suboxide: after administration of bismuth salts by mouth

BACTERIOLOGICAL EXAMINATION

This subject is discussed in Chapter 13 (p. 262).

OVA AND PARASITES

Animal parasites are found more frequently in the tropics or subtropics. Parasites are found also, however, in people who inhabit temperate and cold climates because certain parasites can be transmitted from person to person directly or through food. Today, with increased facilities for travel, there is more intercourse with tropical countries. It seems likely, therefore, that parasites formerly unknown in colder climates will be seen with increasing frequency. This is exemplified by cases of hookworm in returned service men in an area such as New England where hookworm is not endemic.

Relatively few intestinal parasites produce definite symptoms; but others, such as Taenia saginata and Ascaris lumbricoides, cause only vague symptoms. A few parasites, such as Endamoeba coli and Endolimax nana, are not pathogenic, but they must be identified so that they will not be confused with pathogenic forms.

Collection of Specimens:

Stools for examination for ova and parasites are placed in appropriate containers without admixture of urine. Stools following enemas are satisfactory for the examination for ova, helminths, and certain cyst forms.

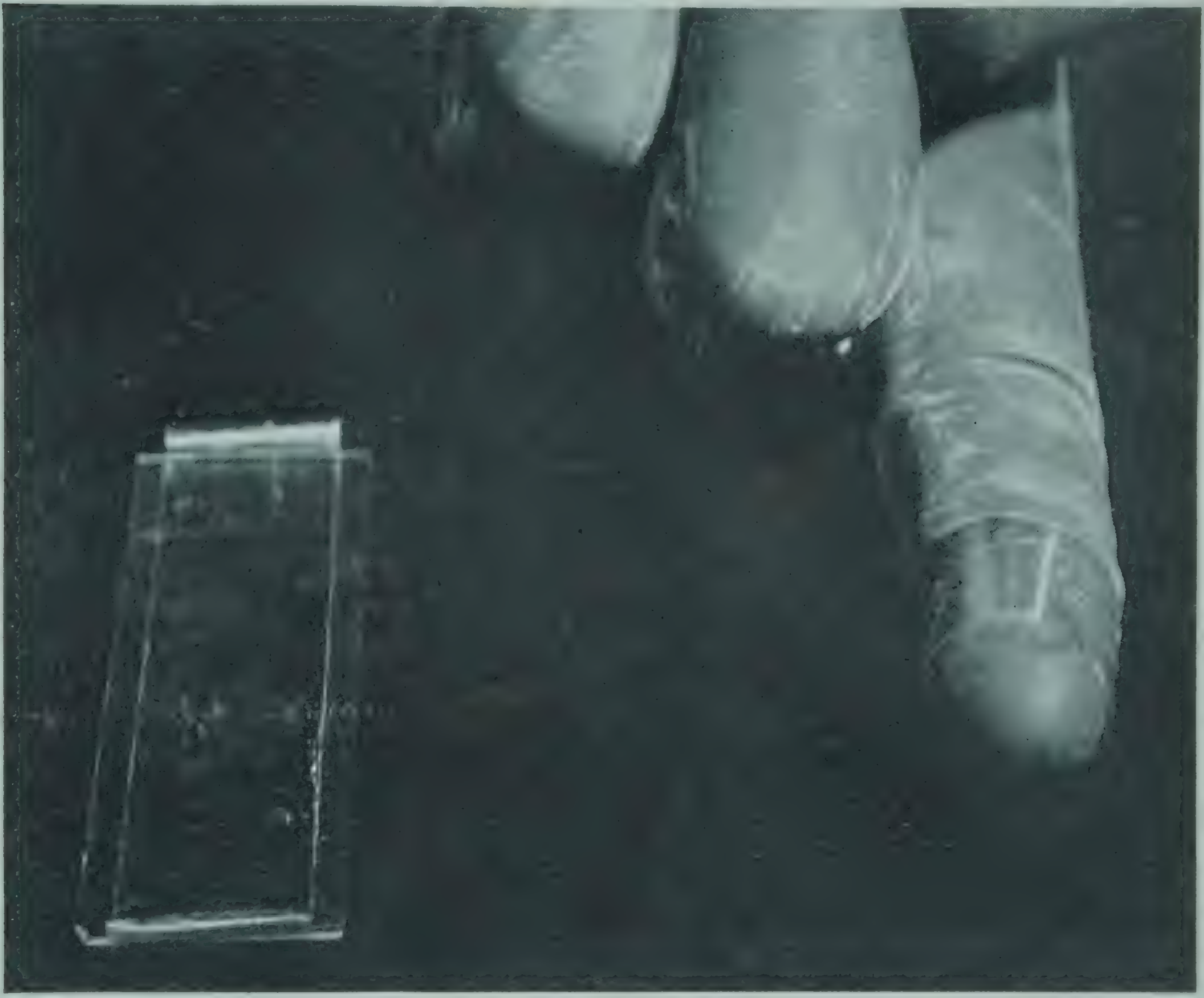

Fig. 1: Scotch-tape method of collecting ova of Enterobius vermicularis from anus.

In examining a stool for Endamoeba histolytica trophozoites, it is necessary to have a fresh, warm specimen. It is preferable to place the material in a jar and to place the jar in a second container filled with warm water. This precaution is necessary in order that the vegetative forms may be seen in motion.

Enterobius vermicularis and ova are rarely seen in the stool unless the person has a heavy infestation. The collection of material from the anus in the morning before defecating yields a greater proportion of positive results. This is done by placing Scotch tape over the gloved finger with the sticky side out. The finger is rolled back and forth across the anus to pick up the ova. Afterward, the tape is attached to a slide, sticky side down, and examined (Fig. 1).

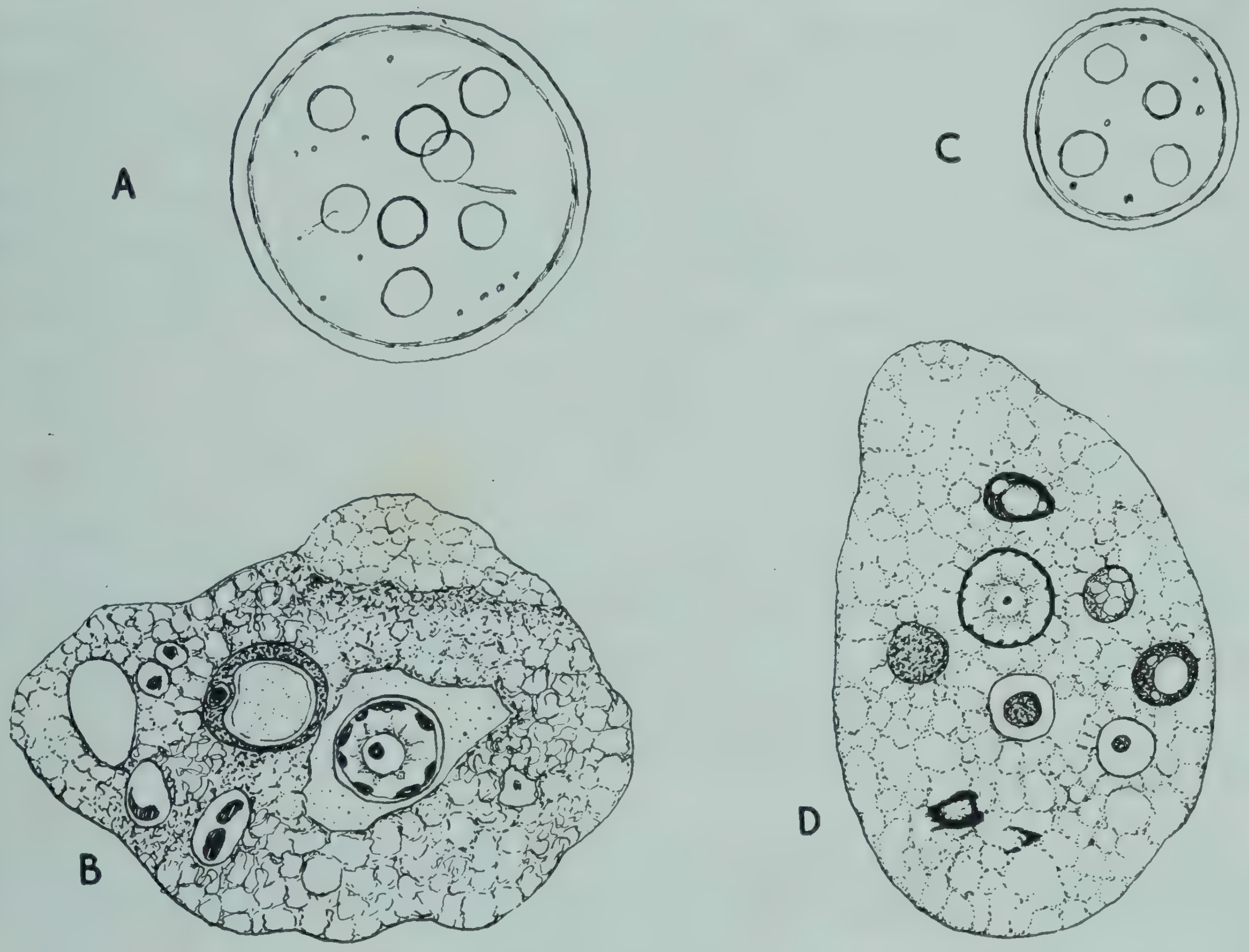

Fig. 2: A: Cyst of Endamoeba coli. *B*: Trophozoite of Endamoeba coli. C: Cyst of Endamoeba histolytica. *D*: Trophozoite of Endamoeba histolytica.

PROTOZOA

Endamoeba Histolytica:

Life Cycle: Only the cyst is infectious. The cyst is transmitted by food, drink, flies, or directly from one person to another. The cyst is ingested and develops into the vegetative form, producing either the acute disease or a carrier state.

Diagnosis: The diagnosis is made by finding the motile, vegetative forms with ingested red-blood cells in the warm, fresh, dysentery stool. The stool should be examined within one-half to one hour after passing so that the amebas will be found alive. Motility cannot be revived by warming a cold stool. The cysts may be identified by the presence of four nuclei in either a liquid or formed stool.

Endamoeba Coli:

Life Cycle: The method of transmission from host to host is similar to that for Endamoeba histolytica. The cysts when ingested hatch in the intestine by binary fission. They live in the lumen of the colon and apparently do not invade the wall as does the histolytical variety.

Diagnosis: This parasite is not pathogenic. The diagnosis is made by finding a sluggishly motile, vegetative form without ingested, red-blood cells. The cyst form with six to eight visible nuclei is characteristic.

Other Amebas:

Other amebas that are not considered pathogenic are as follows: Endolimax nana; Iodamoeba buetschlii; Dientamoeba fragilis, found in the feces; and Endamoeba gingivalis, found in the mouth.

Balantidium Coli:

Life Cycle: The cyst form persists when passed in the feces. It is transmitted by food, water, flies, and from person to person. When the cyst is ingested by a new host, the wall is dissolved, and a ciliated form comes out. The ciliated form undergoes division and bores into the intestinal wall, where it forms ulcers similar to those of Endamoeba histolytica.

Diagnosis: Examination of the feces reveals a large, double-walled cyst with a kidney-shaped nucleus. The motile, ciliated form may be found when the disease is acute.

Intestinal Flagellates:

The parasitic forms found in man are as follows: Giardia lamblia, Chilomastix mesnili, Trichomonas hominis, Retortamonas intestinalis, and Enteromonas hominis.

Life Cycle: The cyst is transmitted by food, water, or flies and is ingested by the new host. The Trichomonas hominis apparently can be transmitted in the vegetative stage. There seems to be no evidence that these parasites in the intestine are pathogenic; however, Giardia lamblia is sometimes found in children with diarrhea.

Diagnosis: The diagnosis of all but Trichomonas hominis is made by finding the charcteristic cyst or, more rarely, the motile form. No cyst form has as yet been found for the Trichomonas hominis.

Helminths

Ascaris Lumbricoides:

Life Cycle: The ovum is transmitted from one host to another by flies, food, and water. Under ideal conditions, the larva in the ovum will develop outside of the body. The ovum when swallowed is bathed in intestinal juices, which stimulates the larva to emerge and molt. At this time, an extraintestinal migration takes place. The larva penetrates into the veins or lymphatics within a few days after ingestion and by way of the right auricle and ventricle goes to the lungs. It invades the alveoli; and after a short period of growth, it returns to the intestine by way of the trachea, epiglottis, and esophagus. After about eight weeks, it reaches adult size in the intestine. The host may be quite sick with Ascaris pneumonia during the pulmonary cycle.

Diagnosis: The diagnosis is made by finding the characteristic, mammillated ova or the adult worm in the feces. The adult worm is sometimes vomited by children. Occasionally, it may be raised in a coughing paroxysm.

Trichuris Trichiuria:

Life Cycle: The ova are transmitted from one host to another by food, flies, insects, domestic animals, or water. The larvae may develop in the ova under ideal conditions outside of the human body. When the ova are ingested, the shells are dissolved; and the larvae proceed to the cecum, where they attach their lashlike, anterior ends to the wall by burrowing.

Diagnosis: The diagnosis is made by finding the oval ova with a clear plug in each end.

Enterobius Vermicularis:

Life Cycle: The ova may be transmitted from the anus to the mouth and thus reinfect the same person. Other hosts may be infected by direct or indirect contact with an infected person. When ingested, the ova yield larvae that escape into the small intestine, molt, mature, and mate. After mating and fertilizing the female, the male worm dies. The female worms attach themselves in the large intestine or descend to attach themselves to the perianal skin, where they dry and burst, spreading the fertile ova about on the skin.

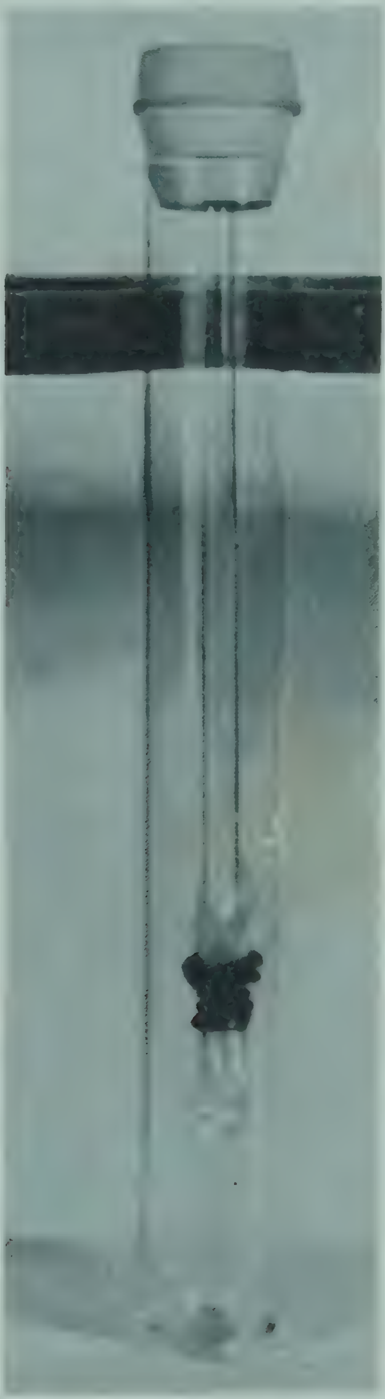

Fig. 3: Swab of National Institute of Health for collecting ova of Enterobius vermicularis from anus.

Diagnosis: The diagnosis is made by finding the adult worm or the planoconvex ova in the material from the anus or vagina. The ova are rarely found in the feces, and the adult worms are found only occasionally unless the infection is massive.

Hookworms (Necator Americanus and Ancylostoma Duodenale):

Life Cycle: When the ova are deposited on warm, moist soil and protected from the sun's rays they hatch in from twenty-four to forty-eight hours. The larvae molt twice and then become filariform larvae, which are infective for human beings. The prospective host, walking barefooted, allows the larvae to penetrate the skin and gain entrance to a blood vessel. The larvae are carried thus to the lungs from which they reach the small intestine by way of the trachea, epiglottis, esophagus, and stomach. They attach themselves to the wall and grow to maturity.

Diagnosis: The diagnosis is made by finding the oval, colorless, segmented ova in the feces. When passed in the feces, the ova have two to eight cells.

Strongyloides Stercoralis:

Life Cycle: The females deposit ova that hatch rhabditic larvae in the intestine. The development after escaping from the intestine in the feces may be direct or indirect: (1) Direct: The rhabditic larvae are transformed into filariform larvae and enter the body through the skin or mucous membrane. (2) Indirect: Sexually mature males and females are formed within thirty hours. The females produce transparent ova, which hatch rhabditic larvae. These molt in three or four days, become filariform larvae, and enter through the skin or mucous membrane. The larvae then enter the venous circulation, which brings them to the lungs. From the lungs, they reach the intestine by way of the trachea, epiglottis, and esophagus and develop into adults.

Diagnosis: The diagnosis is made by finding embryonated ova or rhabditic larvae in the feces.

Clonorchis Sinensis:

Life Cycle: The eggs are ingested by the snail and hatch in the esophagus. After development into cercariae, they leave the snail and swim in the water until they penetrate the muscles of a fish, where they become encysted. The infection is confined to certain Chinese groups who consume the raw fish. The outer capsule is removed by digestion, and the parasite breaks out of the inner capsule. It then migrates to the common bile duct and reaches adult life in the bile capillaries.

Diagnosis: The diagnosis is made by finding opercular ova with the shape of an electric light bulb in the feces.

Fasciolopsis Buski:

Life Cycle: The ova when deposited in suitable fresh water enter a snail. In the snail, sporocysts, mother-and-daughter rediae, and cercarciae develop. The cercarciae leave the snail and encyst upon such water plants as the water caltrop and water chestnut (Trapa natans and Trapa bicornis). These are eaten by laborers who rupture the cysts. The cysts are carried to the intestine and there attach themselves.

Diagnosis: The diagnosis is made by finding the brown, opercular, oval egg or the adult fluke in the feces.

Fasciola Hepatica:

Life Cycle: The ova are deposited in the bile capillaries from which they pass into the intestine. They are passed with the feces into an appropriate environment where the miracidia hatch and enter a snail. They then undergo a change into sporocysts in which rediae develop. Daughter rediae or cercarial larvae are formed. These larvae leave the snail and encyst on water plants. After ingesting the parasitized plants, the larvae pass into the stomach and duodenum. From the duodenum, they pass into the bile capillaries, where they cause cysts and adenomas.

Diagnosis: This is rarely a parasite in man. The diagnosis is made by finding the ova in the feces. These ova simulate those of Fasciolopsis buski.

Schistosoma Mansoni and Japonicum:

Life Cycle: The adult worms are usually found in the vesical plexuses of veins. The female deposits her ova in the smallest veins from which they enter the bladder or feces. The miracidium escapes on reaching the water and penetrates a snail, where it develops into a sporocyst. The daughter sporocyst migrates through the lymph spaces into the liver, where it develops into a cercaria. The cercaria leaves the snail and enters the water, where it can live free for seventy-two hours. If the occasion presents itself, the cercaria enters the skin of a human host, then enters a blood vessel, and ultimately reaches the portal vein, where it becomes an adult.

Diagnosis: The diagnosis is made by finding the characteristic ova in the feces. The ovum of the Schistosoma mansoni is oval, measuring 150 by 65 microns, and has a lateral spine. The ovum of the Schistosoma japonicum is oval, measuring 85 by 60 microns, and has a minute lateral spine.

Taenia Saginata:

Life Cycle: When the ovum leaves the body, it is ingested by the intermediate host, which in this case is cattle. The hexacanth embryo passes through the intestinal wall and goes to the muscles by way of the bloodstream. It goes particularly to the muscles of the head, neck, and heart, where it forms a Cysticercus bovis in the intermuscular connective tissue. It measures about 9 by 5 mm. (⅕ by ⅖ inches) . When improperly cooked meat is eaten, the human host becomes infected. The Cysticercus develops into the adult tapeworm in the intestine.

Diagnosis: The diagnosis is made by finding the ovum or a segment in the feces. The ovum cannot be distinguished from Taenia solium. The segment (proglottis) has from eighteen to thirty uterine branches, which differentiates it from Taenia solium.

Taenia Solium:

Life Cycle: When the ovum is passed in the feces, it is ingested by the intermediate host, a pig. The ovum passes through the intestinal wall into the bloodstream, which carries it to the muscles. In the intramuscular connective tissue, it forms the Cysticercus cellulosae. When improperly cooked pork is eaten, the human host becomes infected. The Cysticercus cellulosae develops into the adult pork tapeworm in the intestine.

Diagnosis: The diagnosis is made by finding the ovum or proglottis in the feces. The ovum cannot be differentiated from that of Taenia saginata. The proglottis has eight to nine uterine branches, which distinguishes it from Taenia saginata.

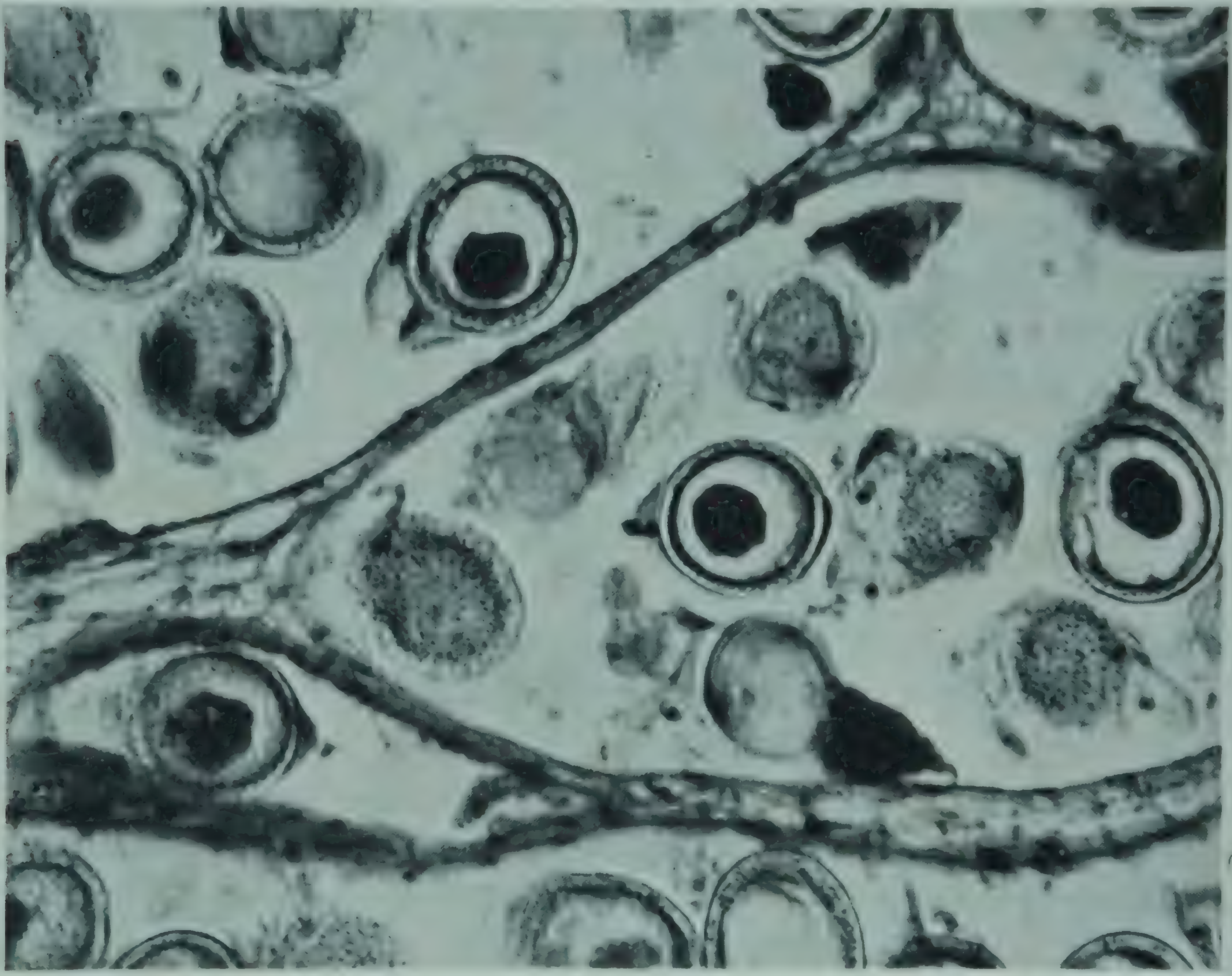

Fig. 4: Ova of Taenia saginata in uterus of segment.

Diphyllobothrium Latum:

Life Cycle: The ovum hatches in water and frees a ciliated embryo after three weeks. This is ingested by a water flea (Cyclops); and in the course of three weeks, develops into a proceroid larva. It migrates from the intestine into the body cavity of the Cyclops. When this Cyclops is ingested by a fresh-water fish, the larva penetrates the intestinal wall and enters the liver, spleen, and muscles by way of the bloodstream. When poorly cooked fish is eaten by a human host, the parasite attaches itself to the intestinal wall by its scolex.

Diagnosis: Examination of the feces reveals the brownish, oval, opercular ovum.

Hymenolepis Nana:

Life Cycle: The ova are transmitted directly from one host to another. No intermediate host is necessary. The cycle takes place within the intestine. The ovum hatches in the intestine, and the embryo penetrates a villus, where it develops into a cysticercoid larva. After a while, the larva ruptures into the intestine, where it attaches itself and develops to maturity.

Diagnosis: Examination of the feces reveals the characteristic ova.

Hymenolepis Diminuta:

Life Cycle: The ova are ingested by a flea, particularly the rat flea, in which they develop into a larval stage. Infestation takes place following ingestion of uncooked food or water containing the dead flea. The majority of cases are found in children under three years of age.

Diagnosis: The diagnosis is made by finding the ova and segments in the feces.

Dipylidium Caninum:

Life Cycle: The ova lodge in the hairs around the anus of the human host. The ovum is ingested by the dog flea, cat flea, flea of man, or the dog louse, in which it develops into a cysticercoid larva in the body cavity. The human host now becomes infected accidentally through the ingestion of water or food that may contain the dead, infected insects.

Diagnosis: The diagnosis is made by finding the ova or a segment in the feces.

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CHAPTER 9

Transudates and Exudates

TRANSUDATES

Formation:

The accumulation of fluid of a noninflammatory nature in a serous cavity is designated as a "transudate." When similar fluid developing from apparently the same causes occurs in the tissues, it is called "edema." Transudates may develop in the pericardium (hydropericardium), pleural cavities (hydrothorax), peritoneal cavity (ascites), scrotum (hydrocele), joints (hydroarthrosis), and subarachnoid space (cerebral edema).

Transudates and edema may be due to the following:

1. Increased pressure in venous capillaries. This may occur in decompensation, kidney disease, and cirrhosis of the liver and as a result, there is an increased capillary permeability, which allows fluid to pass out of the vessels more easily.
2. Increased capillary permeability: This may be due to toxins, prolonged exposure to high temperature, and dietary deficiencies.
3. Hypoproteinemia: In this condition, there is a reduction in colloid osmotic pressure: consequently, edema and transudates will form when the albumin is less than 2.5 gm. per 100 cc.
4. Obstruction of lymphatic vessels: This causes the formation of chylous fluids, and a tumor pressing on the thoracic duct will cause the formation of chylous ascites.

Characteristics:

The fluid is clear and usually of a light straw color unless jaundice or blood is present. If the patient is jaundiced, the fluid may be either amber or golden-yellow. When chyle is present in the fluid, it is white like thin milk. Normally, it does not coagulate immediately but may do so on standing. When the blood is present in the fluid, it coagulates almost immediately because of the fibrinogen.

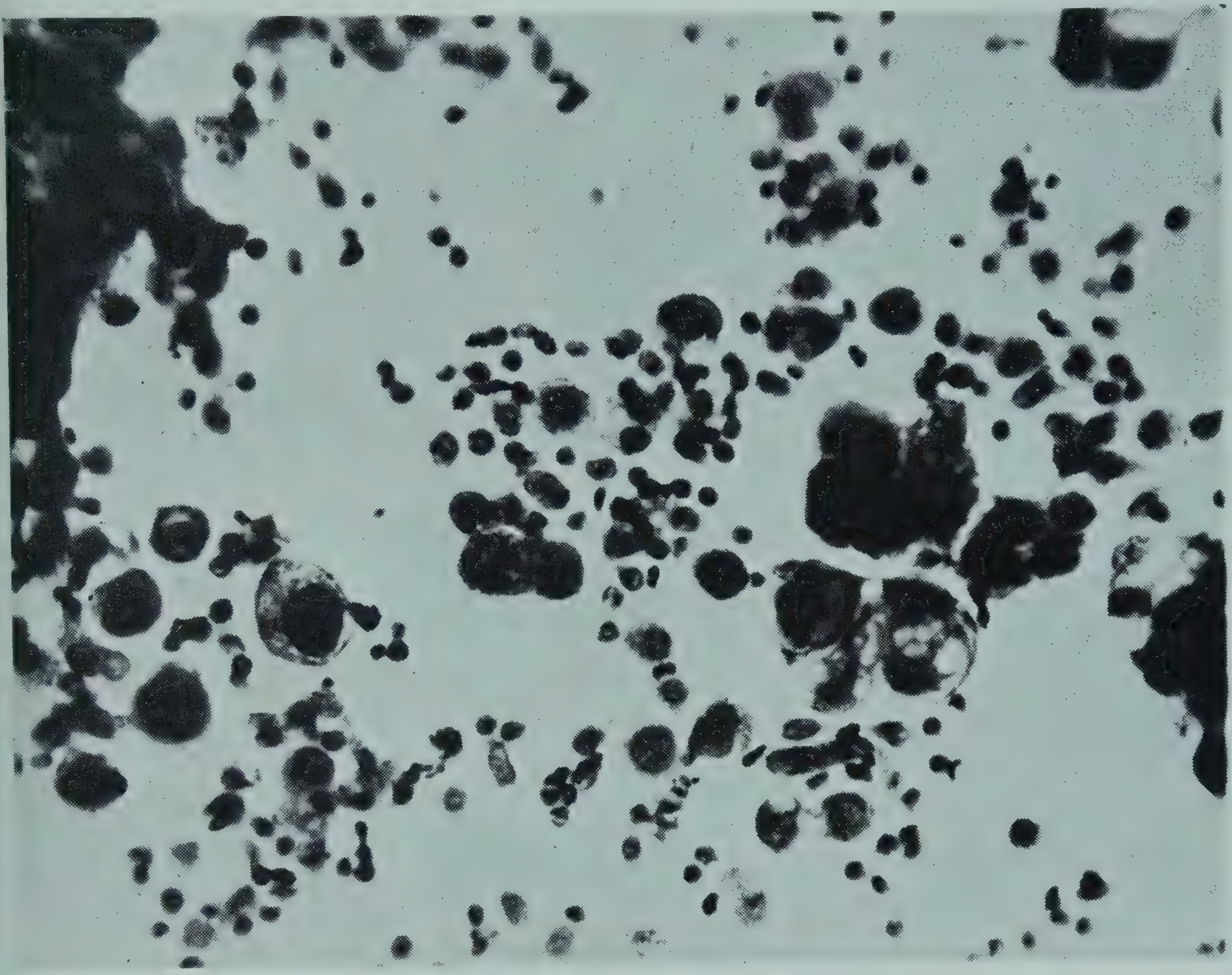

Fig. 1: Tumor cells in pleural fluid.

The specific gravity of transudates is usually less than 1.018. This is because of the low protein level, which is not over 2.5 gm. per 100 cc.

Cells:

In contrast to exudates, transudates contain only a few cells when not contaminated by blood. These cells are lymphocytes and mesothelial cells, the latter from the lining of the cavity in which the fluid has accumulated. In cases of suspected carcinoma, the fluid should be examined for tumor cells by centrifuging and making a cell block of the sediment. The presence of mitosis and cells with nuclei of various sizes and shapes are suggestive of carcinoma. The absence of such cells does not rule out the presence of carcinoma.

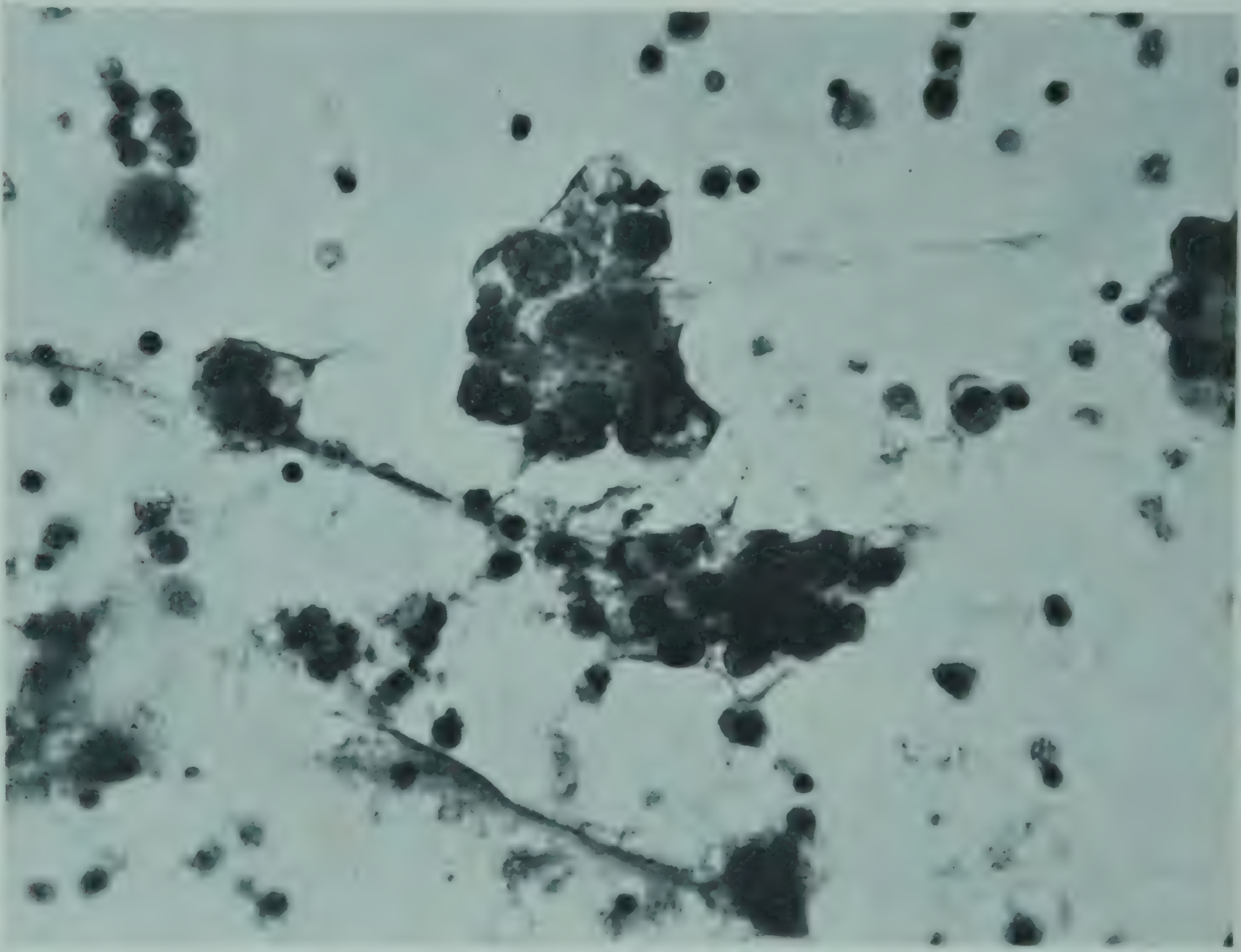

Fig. 2: Tumor cells in ascitic fluid.

Chemical Constituents:

Protein: The total protein of transudates is usually less than 2.5 gm. per 100 cc. Albumin makes up the greatest part of the protein, while globulin is present in only small amounts. In joint fluids, which also contain a substance akin to mucin, protein is present in larger amounts. In pleural fluid due to heart failure, peritoneal fluid secondary to heart failure, or cirrhosis, the protein is decreased and is frequently less than 1 gm. per 100 cc.

Glucose: The glucose is from 80 to 100 mg. per 100 cc. in transudates. This may be of help in differentiating a fluid from an exudate that has a reduced glucose level.

Other Chemical Constituents: The following constituents are also found in transudates, but their determination is not of help clinically:

	Mg. per 100 Cc.
Chloride in terms of sodium chloride	720 to 750
Creatinine	1 to 2
Uric acid	2 to 4
Urea	20 to 30
Calcium	4.5 to 5.5
Phosphorus	2 to 5
Lecithin	20 to 100

Chylous fluids contain finely divided fats varying from 0.05 to 3.85 gm. per 100 cc.

Pseudochylous fluids have the same color as chylous fluids; but the milky appearance is due to lecithin and cholesterol or, in some cases, to finely dispersed albumin.

EXUDATES

Formation:

An exudate is purulent fluid that forms in any body cavity as a result of an inflammatory process. The most common cause is bacterial invasion, but it may be caused by a parasite or by a sterile irritating agent, such as bile or pancreatic juice.

Characteristics:

The color varies from gray to light yellow, depending upon the type of organism and the density of the fluid. It may be green or greenish-yellow when the infecting organism is Pseudomonas aeruginosa. The fluid may be serous or serofibrinous, as well as purulent, in appearance. Coagulation takes place readily because of the presence of fibrinogen.

Exudates infected by Escherichia coli may have a fecal odor, but they are usually odorless or have a sickeningly sweet odor.

The specific gravity is usually more than 1.018 because of the large amount of albumin and the large number of leukocytes.

Cells:

The predominating cells are polymorphonuclear leukocytes. It is not of any clinical aid to count the cells, but a differential count is worth while and should be included in routine examinations. Many leukocytes will show phagocytized bacteria. Mesothelial cells are also present and constitute not more than from 15 to 20 per cent of the total number. Later in the disease, many leukocytes will show evidence of degeneration.

In tuberculous exudates from any of the cavities, lymphocytes predominate, and only a few monocytes and leukocytes are present.

Chemical Constituents:

Protein: The total protein of the supernatant fluid after centrifugation is 3.0 gm. or more per 100 cc. In some cases, it may reach the level of normal blood (6 to 8 gm. per 100 cc.). However, in an exudate caused by the Mycobacterium tuberculosis, as in tuberculous peritonitis, the total protein may be less than 0.5 gm. per 100 cc.

Glucose: The glucose level is much lower than that of transudates because of the glycolytic action of bacteria and leukocytes.

Other Chemical Constituents: The following compounds are also found in exudates and have, with the exception of cholesterol, about the same values as in blood:

	Mg. per 100 Cc.
Chlorides as sodium chloride	570 to 620
Creatinine	1 to 2
Uric acid	2 to 4
Urea	20 to 30
Cholesterol (very variable)	0.05 to 4.5

BACTERIOLOGICAL EXAMINATION

This subject discussed in Chapter 13 (p. 265).

CHAPTER 10

Spermatozoa

ORIGIN

The testes have two functions: (1) production of germ cells, which is known as the "spermatogenic function," and (2) manufacture of the male sex hormone, or the "endocrine function."

The spermatozoa are formed in the seminiferous tubules of the testes from the spermatogonia. These are the youngest cells of the tubules and are seen lying against the basement membrane. Spermatogonia give rise to primary and secondary spermatocytes. The latter divide to form spermatids, which, without further division, evolve into spermatozoa. The spermatogenic cells are stimulated by the follicle-stimulating hormone of the anterior pituitary gland. The spermatozoa plus the secretions constitute the semen.

EXAMINATION

Examination of spermatozoa is of paramount importance in investigating the male partner of a sterile marriage and in cases of alleged rape.

Collection of Specimens:

The specimens for examination are usually collected in a condom. It is preferable to use a clean glass jar because the rubber or the powder in a condom may kill or inhibit the motility of the spermatozoa. If a condom is used, it should be washed and dried thoroughly before using.

It is now believed unnecessary to maintain the semen at body temperature while transporting it to the laboratory. In fact, it should be kept cold previous to examination. The specimen should be examined as soon as possible because the motility is inhibited in a condom.

Macroscopic Examination:

The normal volume of semen is about 4 cc., but it varies greatly in the same person. When first discharged, the semen consists of a mixture of prostatic testicular fluid, which is made up of a tenacious, gelatinous portion and a thin, milky fluid. If it is collected in a bottle, the gelatinous portion adheres to the sides, and the more fluid material may be separated by decanting. Spermatozoa may be found in both portions, with extreme activity in the thin fluid and considerably retarded movement in the gelatinous material.

The color is light gray, and it is normally opaque. The *p*H is always slightly on the alkaline side and averages 7.8.

Microscopic Examination:

This examination falls into three categories: (1) total spermatozoa count, (2) examination for motility, and (3) a stained smear for the determination of abnormal forms.

Total Spermatozoa Count: Macomber and Saunders[1] examined 271 normal and abnormal males and found that the average number of spermatozoa per cubic centimeter was 100,000,000. Belding,[2] in his series, averaged 119,000,000 per cubic centimeter for normal persons and 70,000,000 per cubic centimeter for sterile matings. Marked variations are encountered, however, not only in different persons but also in the same persons at different times. Belding[2] cites one normal person who averaged only 46,000,000 in a series of counts.

It is felt by some that the total number of spermatozoa in an emission is a more accurate method of expressing results than the number per cubic centimeter. The normal range per total emission is from 400,000,000 to 600,000,000.

Examination for Motility: The viscosity of the semen and the temperature at which it is kept influence the motility. The specimen should be kept at 46.4° F. (8° C.) and should be examined within twenty-four hours. In normal, fertile persons, from 10 to 15 per cent of the spermatozoa are inactive.

Smear for Abnormal Forms: A stained smear for the study of abnormal and immature forms is essential. At the same time, the presence or absence of epithelial cells, red-blood cells, or leukocytes is noted. The presence of abnormal spermatozoa and lack of proportion in the heads have been shown by Moench[3] to have a distinct bearing upon fertility. The range between fertile and sterile persons, however, in respect to the total number of abnormal forms is rather narrow; consequently, a detailed study of abnormal types is necessary. A range of up to 20 per cent of abnormal forms is seen in normal persons, but proportions higher than this make fertility less likely.

Abnormalities are seen in the heads, bodies, and tails. Heads may be large, small, atypical in shape, absent, or multiple, and chromatin may be irregularly distributed. Bodies may be large, small, abnormally shaped, absent, multiple, bent, separated from the head, or ruptured. Tails may be large, small, absent, multiple, or irregular.

Examinations in Medicolegal Investigation:

In cases of alleged assault or rape, examination of stains on clothes for spermatozoa can be carried out:

The article or stained area is soaked in physiological salt solution. The sediment is allowed to settle out and is stained. Usually, only the heads of spermatozoa are found, but they are definite proof of the presence of semen. Human spermatozoa, however, cannot be differentiated from animal spermatozoa by this procedure.

Florence Test: This is helpful in detecting old semen stains in clothing:

The cloth is soaked in water and the extract is placed upon a slide. A few drops of the reagent (iodine, 2.54 gm.; potassium iodide, 1.05 gm.;

and distilled water, 30 cc.) are added, and it is examined at once under the microscope. If the stain is dried semen, dark brown crystals in the form of rhombic plates or of needles grouped in clusters will be seen. Negative results are practically conclusive of the absence of semen. The crystals, however, can be obtained from crushed insects dried on cloth and watery extracts of various internal organs; therefore, they are not absolute proof that semen is present.

Hektoen-Precipitin Test: Hektoen[4] has prepared a precipitin test, which is specific not only for semen but also for the particular species.

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CHAPTER 11

Basal Metabolic Rate

Respiration:

Since the determination of the basal metabolic rate is based upon the amount of oxygen utilized, a discussion of respiration and oxygen exchange is advisable. The volume of air inhaled and exhaled during quiet respiration is only about one eighth of the amount that can be exchanged during deep breathing. The air that passes into the lungs and out of the lungs during normal breathing is called "tidal air." This averages about 500 cc. After complete expiration, the normal adult male can inhale, by his greatest effort, about 3000 cc., including the tidal air. This is designated as "complemental air." At the end of ordinary expiration, if an additional forceful expiration is made, about 1000 cc. can be exhaled. This is called "supplemental air." The total amount of air that can be breathed out after maximal inspiration is called the "vital capacity." The following figures are given by Best and Taylor[1] for a healthy adult male of average build:

	Cc.
Tidal air	500
Complemental air (including tidal air)	3000
Supplemental (reserve) air	1000
Vital capacity	4000

Inspired air contains 20.94 volumes per cent of oxygen and 79.02 volumes per cent of nitrogen; expired air contains only 16.3 volumes per cent of oxygen, 4 volumes per cent of carbon dioxide, and 79.7 volumes per cent of nitrogen. This indicates that about 4 volumes per cent of oxygen is taken up by the blood. Most of the oxygen in the blood is in loose combination with hemoglobin. In the lungs, where oxygen tension is high and carbon-dioxide tension is low, the venous blood readily takes up oxygen; but in the tissues, where the reverse is true, the arterial blood gives up oxygen and takes up carbon dioxide. The average oxygen content of arterial blood varies from 16 to 24 volumes per cent, and of venous blood, from 10 to 18 volumes per cent.

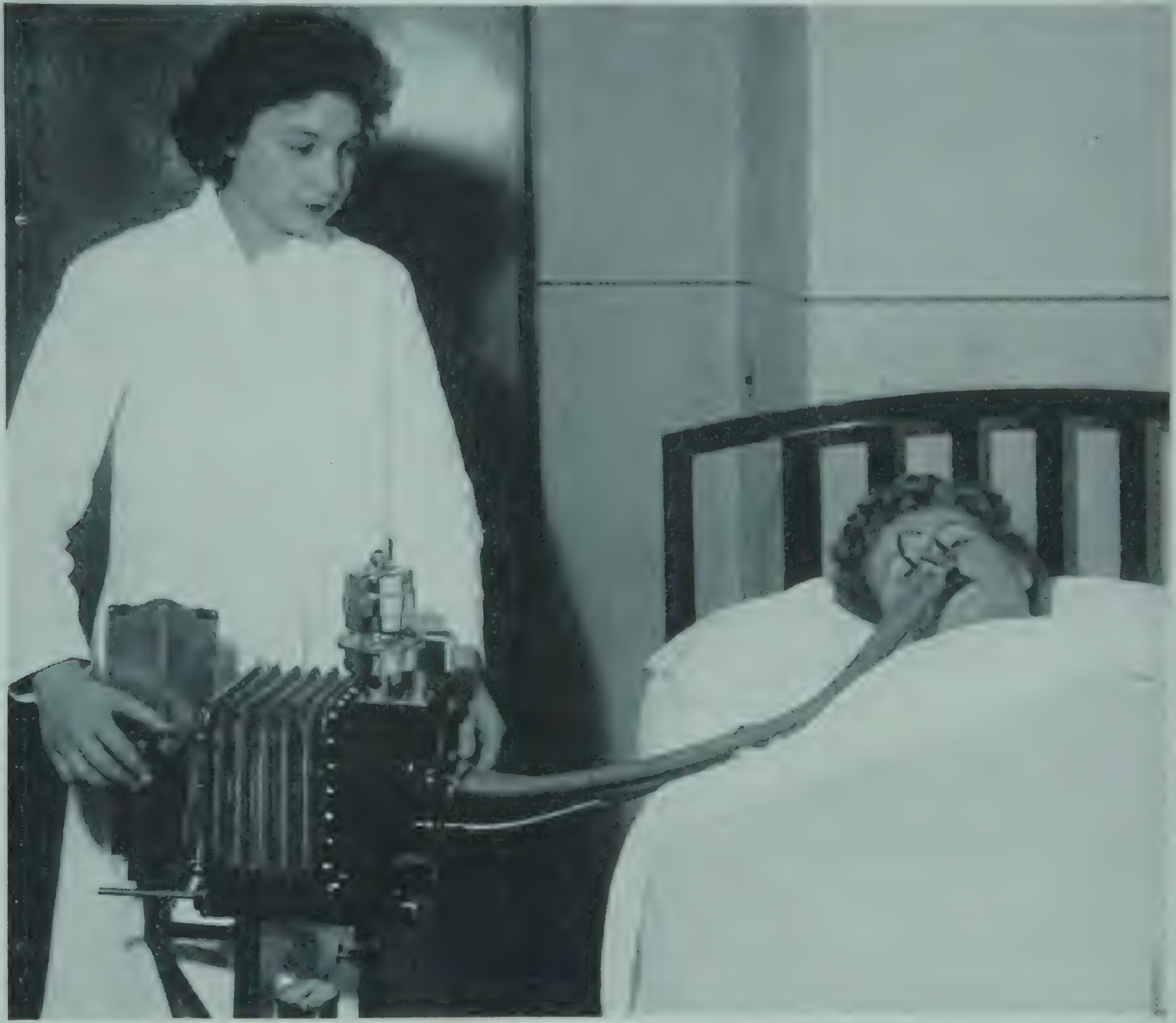


Fig. 1: Basal metabolism test with Jones motor-basal metabolism apparatus. (Courtesy of Jones Metabolism Equipment Co., Boston, Mass.)

Definition:

The basal metabolic rate is the number of Calories given off per hour per square meter of body surface with the patient at complete muscular and mental rest, and fasting for at least fifteen hours. The determining method commonly used in this country is the "spirometric," or "closed," method, in which oxygen utilization is determined in cubic centimeters per minute. The oxygen consumption per square meter of body surface is determined after correcting for age, sex, height, and weight. The results are expressed in per cent and represent the per cent of variation above or below the normal number of Calories per square meter of body surface for a person of a given height, weight, age, and sex.

The normal range is from —10 to +10 per cent, which includes about 75 per cent of normal people. By extending the normal range to from —15 to +15, it includes a total of 95 per cent of normal persons. Most authorities, however, limit the normal range to —10 to +10 per cent.

Physiological Factors Influencing Basal Metabolic Rate:

The following physiological states influence the basal metabolic rate:

Occupation: Laborers and athletes have a higher rate than those leading a sedentary life.

Age: The rate gradually decreases from childhood to old age.

Sex: Males have a slightly higher rate than females of the corresponding age.

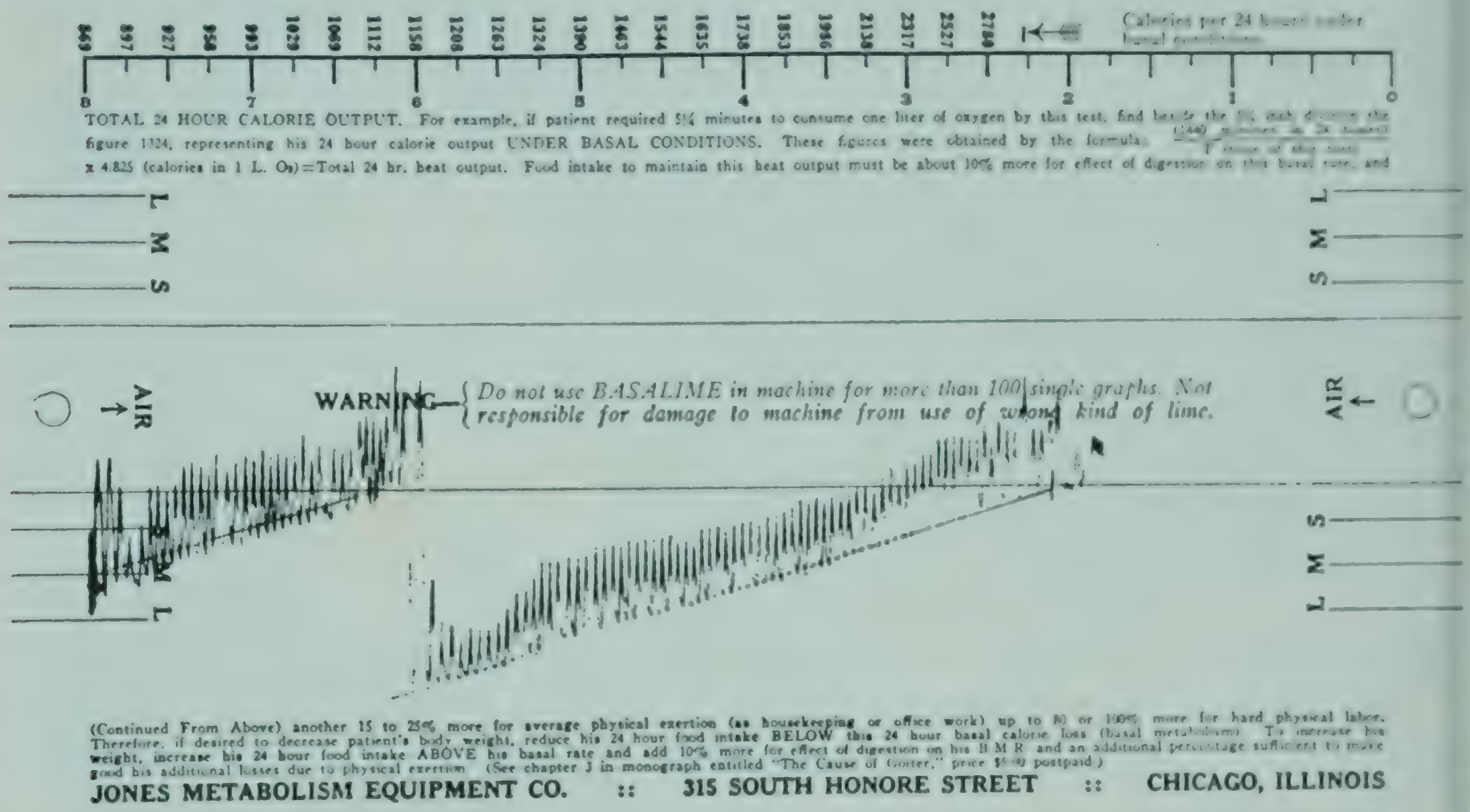
Race and Climate: Some oriental peoples have a slightly lower rate than occidentals living under the same conditions, while others show little difference. The rate is somewhat higher than normal in Eskimos. The rate of white persons living in a tropical climate is usually reduced after a year or more.

Pregnancy: The rate is normal until the beginning of the last trimester, when it is moderately increased. Following delivery, there is a drop to below normal. About six weeks after delivery, the rate becomes normal again, although it may be slightly above normal if lactation is active.

Muscular Exercise: This raises the rate in proportion to the amount of exercise.

Emotional Upsets: Anger and fear will increase the rate appreciably.

Ingestion of Food: This definitely increases the rate by increasing heat production. The influence due to the type of diet (meat or vegetables) is minimal if any.



TOTAL 24 HOUR CALORIE OUTPUT. For example, if patient required 5¼ minutes to consume one liter of oxygen by this test, find beside the [illegible] the figure 1324, representing his 24 hour calorie output UNDER BASAL CONDITIONS. These figures were obtained by the formula: [illegible] x 4.825 (calories in 1 L. O_2) = Total 24 hr. heat output. Food intake to maintain this heat output must be about 10% more for effect of digestion on this basal rate, and

Do not use BASALIME in machine for more than 100 single graphs. Not responsible for damage to machine from use of wrong kind of lime.

(Continued From Above) another 15 to 25% more for average physical exertion (as housekeeping or office work) up to 80 or 100% more for hard physical labor. Therefore, if desired to decrease patient's body weight, reduce his 24 hour food intake BELOW this 24 hour basal calorie loss (basal metabolism). To increase his weight, increase his 24 hour food intake ABOVE his basal rate and add 10% more for effect of digestion on his B M R and an additional percentage sufficient to make good his additional losses due to physical exertion. (See chapter 3 in monograph entitled "The Cause of Goiter," price [illegible] postpaid.)

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Fig. 2: Graph showing respirations and consumption of oxygen. This patient has basal metabolic rate of +16.

Chemical Substances: Caffeine, adrenalin, desiccated thyroid, and α-dinitrophenol raise the rate.

Variations in Barometic Pressure: A reduction in the basal metabolic rate occurs when the barometric pressure falls to one half of an atmosphere. This reduction varies from 5 to 25 per cent.

Conditions Accompanied by Increased Basal Metabolic Rate:

The basal metabolic rate is increased in the following conditions:

1. Thyroid disease
   a. Hyperthyroidism
   b. Exophthalmic goiter
   c. Thyroiditis
   d. Adenoma with hyperthyroidism

2. Other endocrine diseases
    - *a.* Early acromegaly
    - *b.* Pituitary basophilism (sometimes)
    - *c.* Diabetes insipidus
    - *d.* Hyperadrenalism
    - *e.* Diabetes mellitus with severe acidosis
3. Neoplastic processes
    - *a.* Malignancy (in about 30 per cent of cases)
    - *b.* Lymphatic leukemia
    - *c.* Myelogenous leukemia
    - *d.* Malignant granuloma (Hodgkin's disease)
4. Cardiovascular disease
    - *a.* Some cases of essential hypertension
    - *b.* Cardiorenal disease with decompensation
5. Blood dyscrasias
    - *a.* Pernicious anemia
    - *b.* Marked secondary anemia
    - *c.* Erythremia
6. Fever (7 per cent increase for each degree Fahrenheit of rise of body temperature)
7. Convalescence from wasting disease
8. During rapid growth

Conditions with Decreased Basal Metabolic Rate:

The following conditions are associated with a decrease in basal metabolic rate:

1. Thyroid disease
    - *a.* Hypothyroidism
    - *b.* Myxedema
    - *c.* Cretinism
    - *d.* Postthyroidectomy
    - *e.* Thyrogenous obesity
2. Other endocrine diseases
    - *a.* Hypoadrenalism including Addison's disease
    - *b.* Hypopituitarism including pituitary cachexia and adiposal dystrophy
    - *c.* Diabetes mellitus with malnutrition
3. Lipoid nephrosis
4. Shock
5. Malnutrition
6. Nephritis with malnutrition
7. Epilepsy
8. Anorexia nervosa

Conditions with Normal Basal Metabolic Rate:

1. Goiter and adenoma without hyperthyroidism
2. Obesity not related to hypothyroidism
3. Heart disease
4. Nephritis without hypertension
5. Pregnancy (first six months)
6. Menstruation (slight increase in the premenstrual period)

Other Tests:

Reed's Formula: In about 70 per cent of cases, a fairly close agreement is found between the basal metabolic rate as determined by oxygen consumption and the determination by Reed's formula.[2] It may be used in cases where certain physical capacities prevent the usual procedure. The formula is as follows:

Basal Metabolic Rate = 0.683 × (pulse rate + 0.9 of pulse pressure) — 71.5

EXAMPLE: An individual with a pulse rate of 98 and a pulse pressure of 65:

Basal Metabolic Rate = 0.683 × (98 + 0.9 × 65) — 71.5
Basal Metabolic Rate = + 35 per cent

Gasometric Methods: In using the gasometric methods, the subject inhales atmospheric air and exhales into a bag or into a spirometer. In the Douglas-bag method, the subject breathes into a rubber-lined bag with a capacity of 100 liters. It is fitted with a tube and mouthpiece. The valves are arranged so that the inspired air is taken from the room, and the expired air is forced into the bag. A three-way valve is located at the junction of the tubing with the bag. With the valve in one position, the subject can exhale into the outside air and thus become accustomed to the apparatus before starting the test. When the test is started, the valve is adjusted so that the expired air enters the bag. At the end of the test, the bag is closed by turning the valve off. The mouthpiece is removed, and the end of the tubing is connected with a gas-analysis apparatus. The valve is opened, and the bag is compressed, thus forcing the contents through the apparatus. This method has the advantage that it can be used during exercise.

Tissot's method is similar in principle to that of the Douglas-bag method, except that the subject expires into a bell-type spirometer with a capacity of 100 liters or more. The volume of expired air can be determined by the rise in the spirometer cylinder. Both tests are run for a period of ten minutes.

The amount of oxygen absorbed per minute is computed, and from this the number of Calories produced per hour is computed.

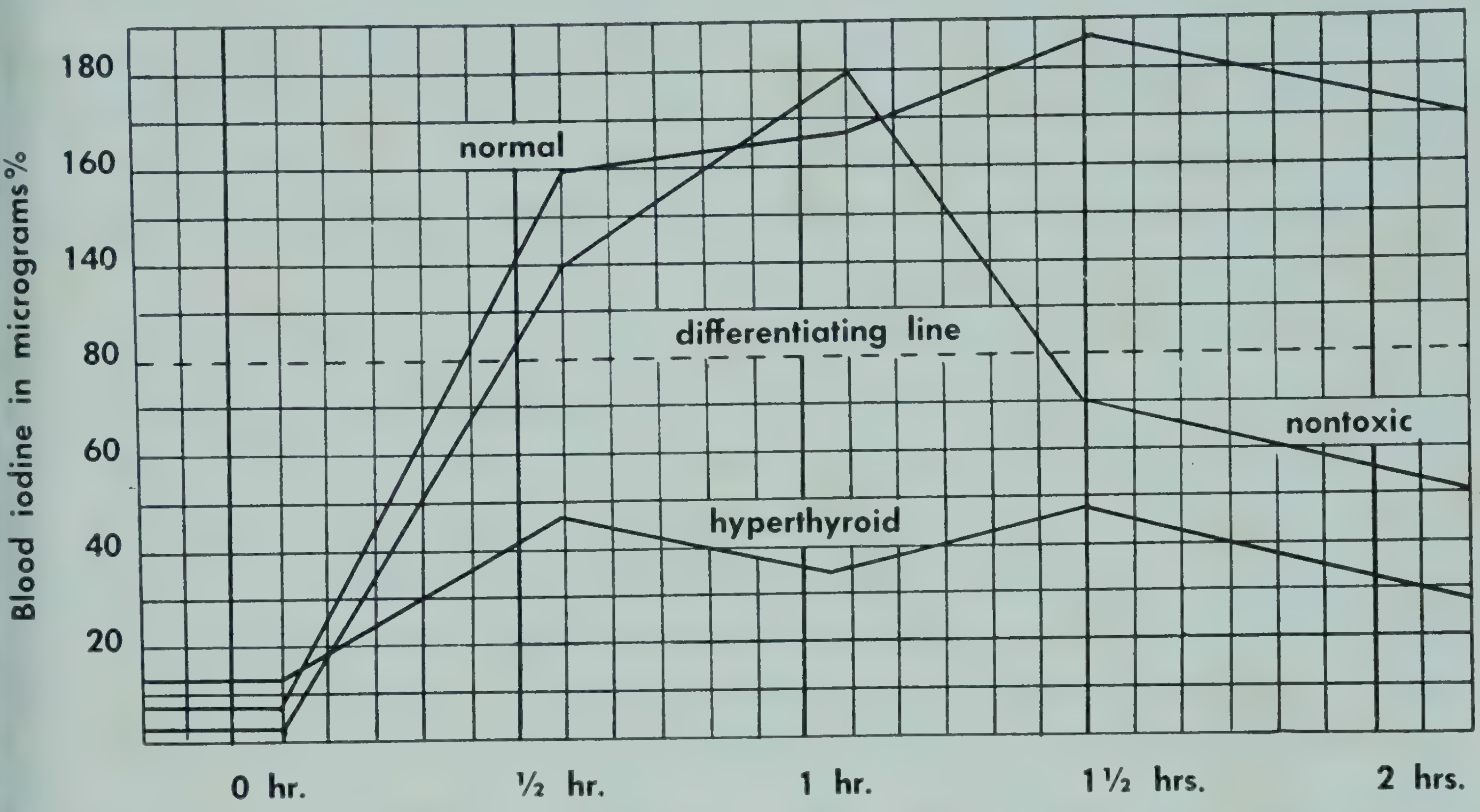

Fig. 3: Iodine-tolerance tests.

Iodine-Tolerance Test: An iodine-tolerance test was introduced by Perkin and co-workers[3] as a means of differentiating between cases of toxic and nontoxic thyroid conditions. The normal blood iodine ranges from 8 to 16 micrograms (1 microgram $=\frac{1}{1000}$ of a milligram) per 100 cc. of blood. Variations in blood-iodine levels, however, do not parallel hyperthyroid states because some persons have normal levels in spite of severe toxicity. The iodine-tolerance test indicates more clearly a condition of hyperthroidism.

PROCEDURE:

The patient undergoing the test should be fasting and should not have taken iodine in any form for at least a week. A fasting blood-iodine determination is made, and from 0.3 to 0.5 cc. (5 to 8 minims) of strong iodine solution is given in milk. Blood-iodine determinations are made each half hour for two and one-half hours.

In normal persons, the thyroid gland has little need for iodine; therefore the blood level increases markedly. It rises from 10 to 160 to 170 micrograms per 100 cc. in one-half hour and at the end of the test drops only to 150 micrograms. In hyperthyroidism, on the other hand, the curve rises from about 15 to only 40 micrograms at the end of an hour. This suggests that the thyroid gland and body tissues are in need of iodine and rapidly absorb it from the blood. Excessive excretion must be considered as a possible means of losing iodine from the blood. Elmer[4] observed, however, that the same amount of iodine is excreted by normal persons and those with hyperthyroidism.

Protein-Bound Iodine: The determination of protein-bound iodine in the plasma is apparently a more accurate means of evaluating the state of thyroid activity than is the level of total iodine or inorganic iodine. According to Bassett, Coons, and Salter,[5] the determination of protein-bound iodine ("P" iodine) is a practical and useful method for clinical investigation of thyroid activity because it affords a roughly quantitative measurement of the circulating thyroid hormone. These authors give a normal range of from 4.6 to 5.9 micrograms per 100 cc. with a mean of 5.2 micrograms per 100 cc. They state, however, that too much stress should not be placed upon absolute values since results vary with different technics. Each laboratory should state its own range of normal values, based upon the technic in use.

Salter, Bassett, and Sappington[6] analyzed these results in one hundred cases of suspected thyroid disturbance. They found that the "P" iodine was consistently reduced in hypothyroidism and myxedema, ranging from 0.8 to 3.9 micrograms. In hyperthyroidism, the "P" iodine was increased and reached as high as 24.2 micrograms per 100 cc.

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CHAPTER 12

Tests for Liver and Biliary Function

PHYSIOLOGY OF LIVER FUNCTION

In order to understand liver-function tests, it is necessary to know the physiology of the liver with its multiplicity of functions. The functions are summarized as follows:

- Metabolism of:
    - Carbohydrate
    - Protein
    - Fat
- Production of:
    - Bilirubin
    - Fibrinogen
    - Plasma albumin and globulin
    - Prothrombin
    - Heparin
    - Vitamin A
- Storage of:
    - Antianemic factor
    - Iron and copper
    - Vitamins A, D, and K
- Detoxification of:
    - Indole, skatole, and phenol
    - Salicylic acid
    - Menthol
    - Camphor, and so on
- Excretion of:
    - Bilirubin
    - Phosphatase
    - Dyes

Carbohydrate Metabolism:

Glucose is brought to the liver from the intestine by means of the portal circulation. The excess, which is not needed for immediate use, is stored in the liver as glycogen (glycogenesis). The liver also forms glycogen from protein and lactic acid, and possibly from fat. This supply of glycogen in the liver constitutes a reserve supply with which to maintain a normal glucose concentration in the blood. Glycogen is also stored in the tissues, and this glycogen may be used immediately for tissue metabolism.

Because of the function of the liver in storing glycogen, glucose, levulose, galactose, and lactic acid, tolerance tests have been devised to test the efficiency of the liver.

Protein Metabolism:

In metabolism of protein it has been demonstrated that the liver is normally the only site of deamination of amino acids. The presence of amino acids in the blood in extensive liver disease is due to the inability of the liver to deaminize the amino acids, which accounts for the presence of the amino acids, leucine and tyrosine, in the urine in acute yellow atrophy. The liver also forms urea from ammonia derived from the amino acids. In fact, urea formation parallels deamination, and the amount of urea formed depends upon the efficiency of deamination. It also has an important role in the destruction of uric acid. The determination of urea and uric-acid blood levels is not a satisfactory method of determining liver damage because of the extensive disease that is necessary before there is (1) a decrease in the blood level of urea and (2) an increase in the blood level of uric acid. The blood level of uric acid, however, appears to be a fairly satisfactory guide in preeclamptic toxemia and eclampsia.

Both the liver and kidney detoxify benzoic acid by combining it with glycine to form hippuric acid. This is the basis for the hippuric-acid, liver-function test.

Fat Metabolism:

The liver takes part in the metabolism of fat by excreting bile, which activates pancreatic lipase and emulsifies fats. The bile salts also form soluble compounds with fats.

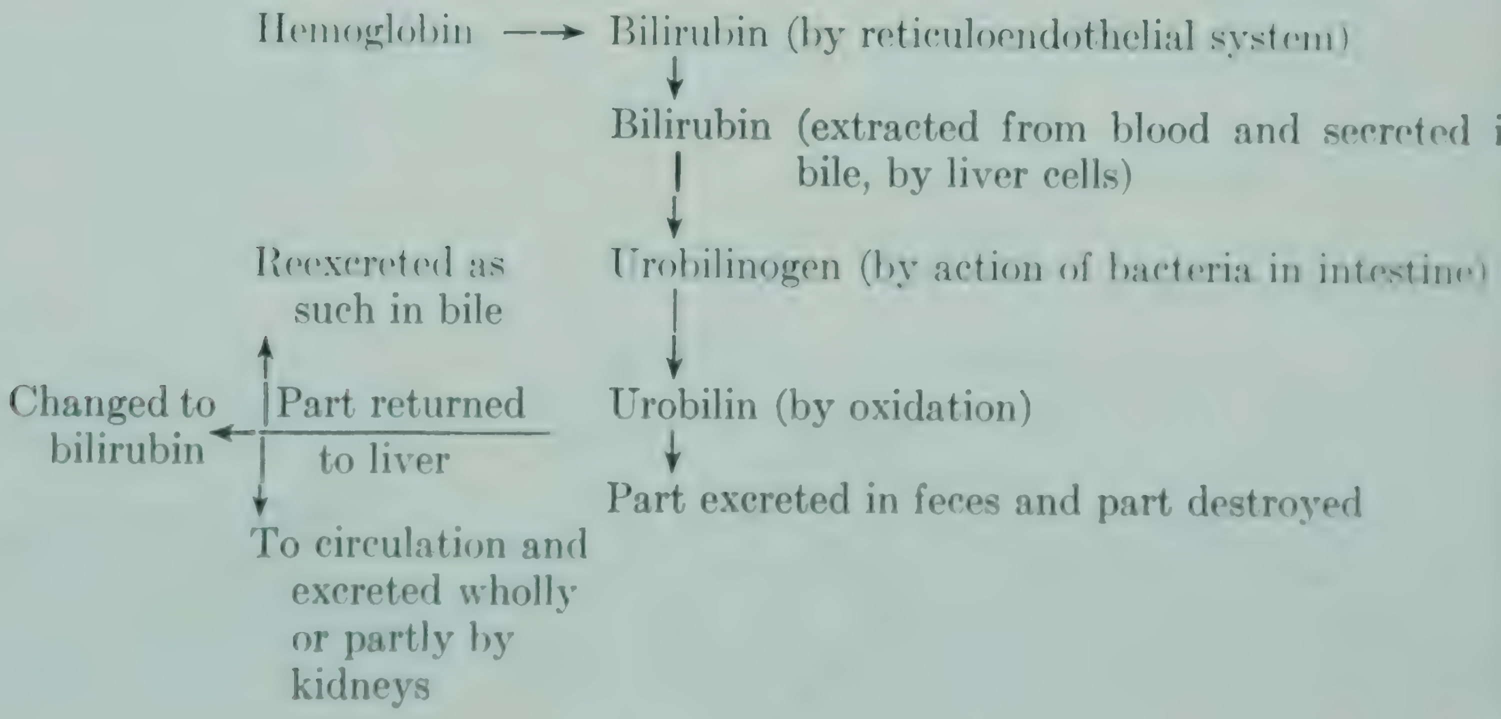
Intrinsically, the liver plays an important role in fat metabolism[1] as evidenced by the following: (1) Fatty acids combined as liver phosphatides and glycerides are more unsaturated than fatty acids found in other tissues. (2) Under a great variety of conditions, neutral fat or cholesterol esters may accumulate in the liver to a much greater degree than in any other tissue. (3) The liver is the principal, if not the only, site of ketone-body formation. (4) The rate of phospholipid turnover in the liver is more rapid than in any other tissue, with the possible exception of the intestinal mucosa during fat absorption.

The liver may be involved to some extent in the synthesis and destruction of cholesterol. It probably synthesizes cholesterol esters and maintains a constant ratio between free and esterified cholesterol in the blood.

Lipotropic factors, such as choline, casein, and betaine, are important in the diet because, in their absence, fat accumulates in the liver.

Production of Bilirubin:

Bilirubin is formed from hemoglobin by the cells of the reticuloendothelial system in the liver (Kupffer's cells), spleen, and bone marrow. The liver cells themselves appear to function only in taking the bilirubin from the bloodstream and secreting it into the bile capillaries. When the bilirubin enters the intestine, it is acted upon by bacteria, which convert it into urobilinogen. Then, by oxidation, urobilinogen is changed to urobilin. Most of the urobilin is excreted in the feces, but some is destroyed and some returned to the liver by the portal system. Bile-pigment formation may be outlined as follows:


Hemoglobin
Bilirubin (by reticuloendothelial system)
Bilirubin (extracted from blood and secreted in bile, by liver cells)
Urobilinogen (by action of bacteria in intestine)
Reexcreted as such in bile
Changed to bilirubin
Part returned to liver
Urobilin (by oxidation)
Part excreted in feces and part destroyed
To circulation and excreted wholly or partly by kidneys

Production of Fibrinogen:

Evidence points to the liver as the site of fibrinogen production, since the concentration of this substance in the blood is reduced by liver damage or hepatectomy. When the liver returns to a healthy state after injury has been induced by such an agent as phosphorus, the fibrinogen level also returns to normal.

Production of Albumin and Globulin:

In the adult, the plasma proteins may originate from one or more of the following sources: (1) disintegrating red- and white-blood cells; (2) general tissue cells; (3) reticuloendothelial cells of the spleen, bone marrow, and so on; and (4) the liver (in fact, the liver is believed to play a major role in the production of the albumin and globulin fractions of the blood).

Production of Prothrombin:

The liver is probably the chief source of prothrombin, but the production is governed by vitamin K. There is evidence that some prothrombin is produced by the bone marrow.

Production of Heparin:

McLean[2] discovered that certain crude extracts of liver inhibited the coagulation of blood. Later Howell and Holt[3] obtained a powerful anticoagulant from liver extracts and to this they gave the name of "heparin." A unit of heparin is the quantity of material that will prevent the clotting of 1 cc. of cat's blood for twenty-four hours when kept in the cold. The anticoagulant activity of heparin is due to its ability to inactivate thrombin. Only minute amounts of heparin are present in the circulation, and it appears not to be responsible for the fluidity of blood in humans.

Production of Vitamin A:

The three types of carotene, alpha, beta, and gamma, and possibly other plant pigments, are the precursors of vitamin A. When taken in food, carotene, or provitamin, is converted into vitamin A in the liver, where it is probably stored in Kupffer's cells.

Storage of Vitamins:

In addition to vitamin A, vitamins D and K are believed to be stored in the liver (see p. 537).

Storage of Antianemic Factor:

According to Castle's work, an intrinsic factor secreted by the gastric mucosa acts upon the extrinsic factor found in certain food.

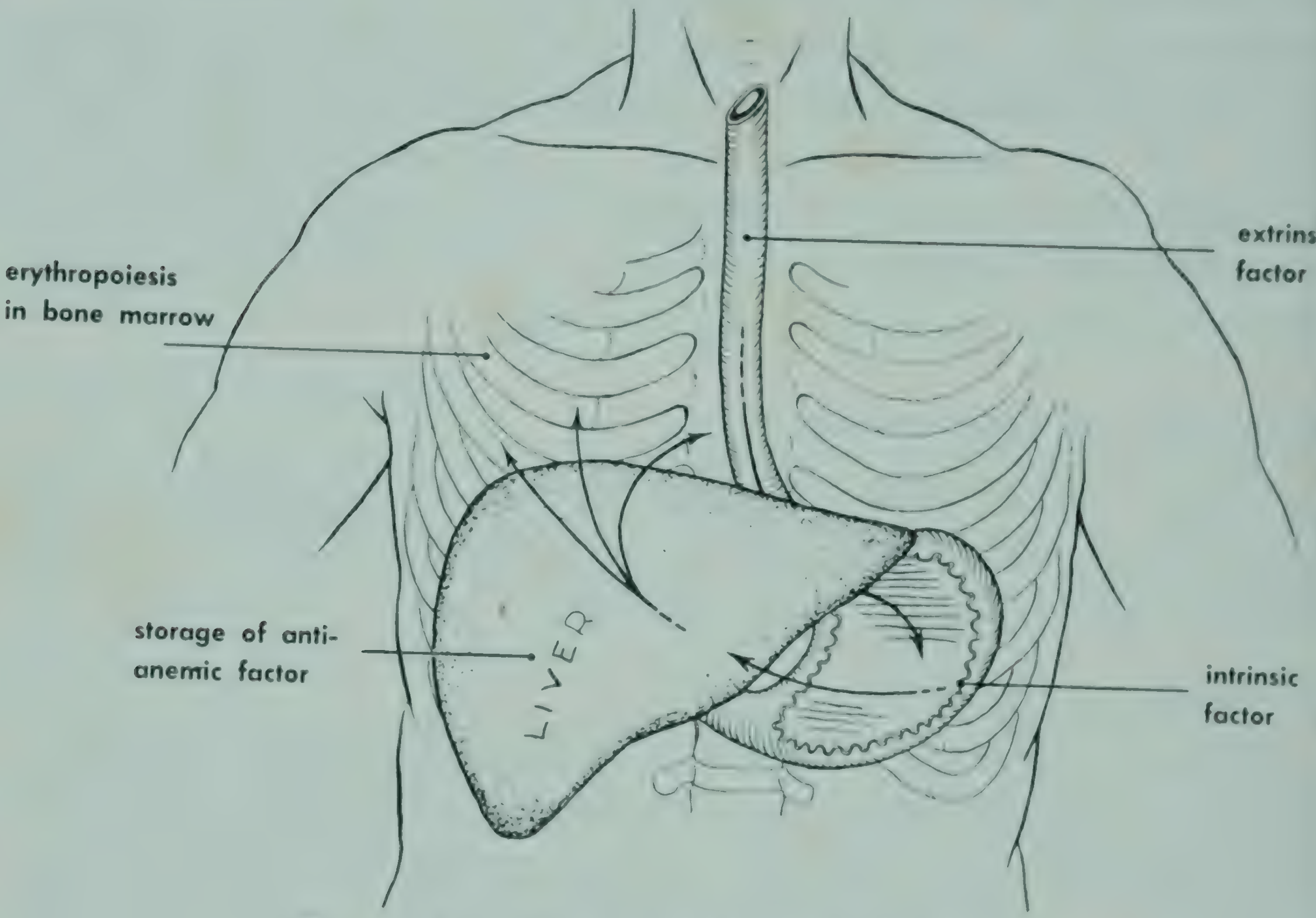

extrinsic factor
erythropoiesis in bone marrow
storage of anti-anemic factor
LIVER
intrinsic factor

Fig. 1: Formation of erythropoietic factor.

The two factors are thus concerned with the production of the antianemic factor, which is stored in the liver and possibly other organs. The antianemic factor, most of which is stored in the liver, acts upon the bone marrow to maintain normal erythropoietic activity (see p. 160).

Storage of Iron and Copper:

Iron is absorbed into the bloodstream mostly from the upper part of the small intestine. It disappears rapidly and is stored in the liver and, to a lesser extent, in the spleen and kidneys.

Copper acts as a catalyst in some stage of hemoglobin synthesis but does not enter into the hemoglobin molecule. The liver is the main storehouse for copper, and it is present in the blood in very small amounts (from 0.1 to 0.5 mg. per 100 cc.).

Detoxification Function:

Toxic substances entering the body by way of the intestinal tract are believed to be detoxified in the liver by oxidation and conjugation into less toxic or nontoxic products. Indole, skatole, and phenol are absorbed into the bloodstream but are detoxified in the liver by conjugation with sulfuric acid and potassium, or with glucuronic acid. Indole and skatole are converted into indoxyl; this, for the most part, combines with sulfuric acid and potassium to form indoxyl sulfate of potassium (indican), which is excreted in the urine.

Other toxic products, such as salicylic acid, menthol, and phenol, are detoxified by conjugation with glucuronic acid.

Excretion of Bilirubin:

After the formation of bilirubin by Kupffer's cells of the liver and the reticuloendothelial cells of the spleen and bone marrow, it is taken out of the bloodstream by the liver cells and excreted into the biliary canaliculi. It is carried through the biliary system to the duodenum, where it is subsequently changed to urobilinogen by the action of bacteria.

Excretion of Phosphatase:

Alkaline phosphatase is believed to be a product of the osteoblasts, proliferating cartilage cells, and the cells of the inner layer of the periosteum. The blood level of alkaline phosphatase is from 1.5 to 4 Bodansky units per 100 cc. The liver excretes phosphatase; consequently, when there is jaundice due to obstruction or liver damage, the phosphatase level is increased.

Excretion of Dyes:

The liver is concerned in the excretion of certain dyes that are utilized as liver-function tests. The most successful of these is bromsulfalein, which is less toxic than the other dyes (see p. 232).

CLASSIFICATION OF TESTS

The tests for liver and biliary function may be classified as follows:

1. Tests based upon excretory function
    - *a.* Bromsulfalein test
    - *b.* Bilirubin-tolerance test
    - *c.* Rose-bengal test
    - *d.* Phenyltetraiodophenolphthalein (isoiodeikon) test
2. Tests based upon metabolic function
    - *a.* Galactose-tolerance test
    - *b.* Levulose-tolerance test
    - *c.* Glucose-tolerance test
    - *d.* Hippuric-acid test
    - *e.* Response of plasma prothrombin to ingestion of vitamin K
3. Tests showing changes in blood secondary to liver damage or biliary obstruction
    - *a.* Cephalin-cholesterol-flocculation (Hanger's) test
    - *b.* Thymol-turbidity test
    - *c.* Thymol-flocculation test
    - *d.* Takata-Ara test
    - *e.* Icterus index
    - *f.* van den Bergh reaction
    - *g.* Quantitative-bilirubin test
    - *h.* Alkaline-phosphatase test
    - *i.* Plasma proteins (albumin-globulin ratio)
    - *j.* Free and total cholesterol
    - *k.* Glucose level
    - *l.* Colloidal-gold test
    - *m.* Vitamin-A level of plasma
4. Tests showing changes in urine and feces secondary to liver damage or biliary obstruction
    - *a.* Urobilinogen test
    - *b.* Bilirubin test
    - *c.* Tyrosinuria test

TESTS BASED UPON EXCRETORY FUNCTION

Bromsulfalein Test:

This test is based upon the ability of the liver cells to take up and excrete the bromsulfalein dye after intravenous injection. Some of the dye may be destroyed by Kupffer's cells. The dose is 5 mg. of bromsulfalein given intravenously. At the end of thirty minutes and at the end of one hour, withdraw 5 cc. of blood for determination of the amount of dye in the blood.

The complete absence of dye or the presence of only a trace indicates normal liver function. The retention of from 20 to 40 per cent of the dye in the blood indicates slight impairment of liver function; from 50 to 80 per cent, moderate damage; and above 90 per cent, very severe damage. In acute and chronic hepatitis with hepatocellular damage, there is an abnormal retention of the dye in the blood. In complete obstruction of the common bile duct, the amount of retention of bile in the blood increases with the amount of bilirubin; thus, the test is of little value. In chronic, incomplete obstruction with liver-cell damage, however, the dye may be retained in the blood out of all proportion to the level of bilirubin. In such a case, the result of the test is significant.

Bilirubin-Tolerance Test:

This test is based upon the excretion of bilirubin by the liver cells following the intravenous injection of a bilirubin solution (1 mg. per kilogram of body weight in 15 cc. of one-tenth molar solution of sodium carbonate).

At the end of four hours, the blood does not show a retention of more than 6 per cent in normal persons. Retention of more than 6 per cent in the blood is seen in acute and chronic hepatitis with hepatocellular damage, cirrhosis, and extensive carcinoma.

Rose-Bengal Test:

The basis of this test is the excretion of rose bengal by the liver into the intestinal tract after the intravenous injection of 10 cc. of the dye. This dye is toxic for some persons, and it colors the feces red.

Normally, the concentration of dye in the blood should be 50 per cent less in the six-minute specimen than in the two-minute specimen. If more than 50 per cent of the dye is retained in the blood, it indicates impairment of liver function.

Phenyltetraiodophenolphthalein (isoiodeikon) Test:

This test was originally designed to be combined with x-ray studies of the gallbladder. The basis of it is the excretion of the dye into the bile by the liver after intravenous injection of 1 cc. per kilogram of body weight of a sterile, 1 per cent solution of phenyltetraiodophenolphthalein.

Normally, from 10 to 15 per cent of the dye is retained in the blood thirty minutes after injection. If more than 20 per cent is retained, it indicates impairment of liver function. Retention of more than 50 per cent indicates rather severe liver damage.

TESTS BASED UPON METABOLIC FUNCTION

Galactose-Tolerance Test:

The galactose-tolerance test is based upon the ability of the liver to store glycogen following the ingestion of 1 gm. of galactose per kilogram of body weight in water flavored with lemon.

Normally, the excretion of galactose in the urine totals less than 3 gm., and the blood level in two hours is from 30 to 120 mg. per 100 cc.

This test is of no value in chronic liver disease or in the absence of jaundice. It may show increased urinary excretion in extrahepatic, obstructive jaundice. There is an increased excretion (6 gm. or more) in serious liver damage.

Levulose-Tolerance Test:

This test is based upon the ability of the liver to transform levulose into glycogen following the ingestion of 50 gm. of the sugar in 200 cc. of water. Three half-hour specimens of blood are examined for levulose.

Normally, there is less than 20 mg. per 100 cc. in the first hour and less than 8 mg. per 100 cc. at the end of the second hour.

This test is of no value in the presence of diabetes mellitus. The test is abnormal if there is an increase to 35 mg. per 100 cc. or more at any time during the two-hour period or if there is a delay in returning to normal beyond the two-hour period. It is of value in severe liver damage but gives less significant values in obstructive jaundice and cirrhosis.

Glucose-Tolerance Test:

The glucose-tolerance test is based upon the inability of the liver to store glycogen following the ingestion of 50 to 100 gm. of glucose.

Venous blood contains 140 to 160 mg. of glucose per 100 cc. within one hour in normal persons. Higher blood-sugar values in one to three hours are indicative of liver disease. However, the test is not considered helpful in evaluating liver damage because of factors outside the liver concerned with glucose metabolism.

Hippuric-Acid Test:

This test is based upon the ability of the liver to conjugate ingested benzoic acid and glycine into hippuric acid with excretion in the urine.

When a normal adult is given 6 gm. of sodium benzoate by mouth, the excretion is from 4.2 to 5.9 gm. of hippuric acid in four hours. When 1.77 gm. is administered intravenously, the excretion is from 1 to 1.5 gm. of hippuric acid in one hour.

In severe hepatitis, the excretion of hippuric acid is very low, and there is some reduction in catarrhal or epidemic jaundice. It may also be decreased in nephritis, anemia, and cachexia.

Response of Plasma Prothrombin to Injection of Vitamin K:

Lord and Andrus[4] recommend consideration of a plasma-prothrombin response to an intramuscular injection of menadione as a differential diagnostic procedure in cases of jaundice.

A sample of blood is taken for the determination of the initial level of plasma prothrombin, and 2 mg. of menadione is injected intramuscularly. At the end of twenty-four hours, a second sample of blood is examined for prothrombin.

If a rise of 10 per cent or more in the level of plasma prothrombin has taken place, the patient is considered to have extrahepatic jaundice, and no more determinations are necessary. If a rise of less than 10 per cent occurs, however, it is necessary to determine the plasma-prothrombin level at either forty-eight or seventy-two hours after the injection. If the rise is then 15 per cent or more over the initial level, the patient probably has extrahepatic jaundice; if less than 15 per cent, the jaundice is considered to be of intrahepatic origin and associated with liver damage. Lord and Andrus have found that every case of jaundice in which the initial level of plasma prothrombin was 80 per cent or more has been one of extrahepatic jaundice.

TESTS SHOWING CHANGES IN BLOOD SECONDARY TO LIVER DAMAGE OR BILIARY OBSTRUCTION.

Cephalin-Cholesterol-Flocculation Test (Hanger's Test):

Hanger[5] noted that emulsions made from sheep-brain cephalin and cholesterol will flocculate in the presence of serum from patients with active hepatitis and disintegration of liver parenchyma, whereas they remain stable with normal serum.

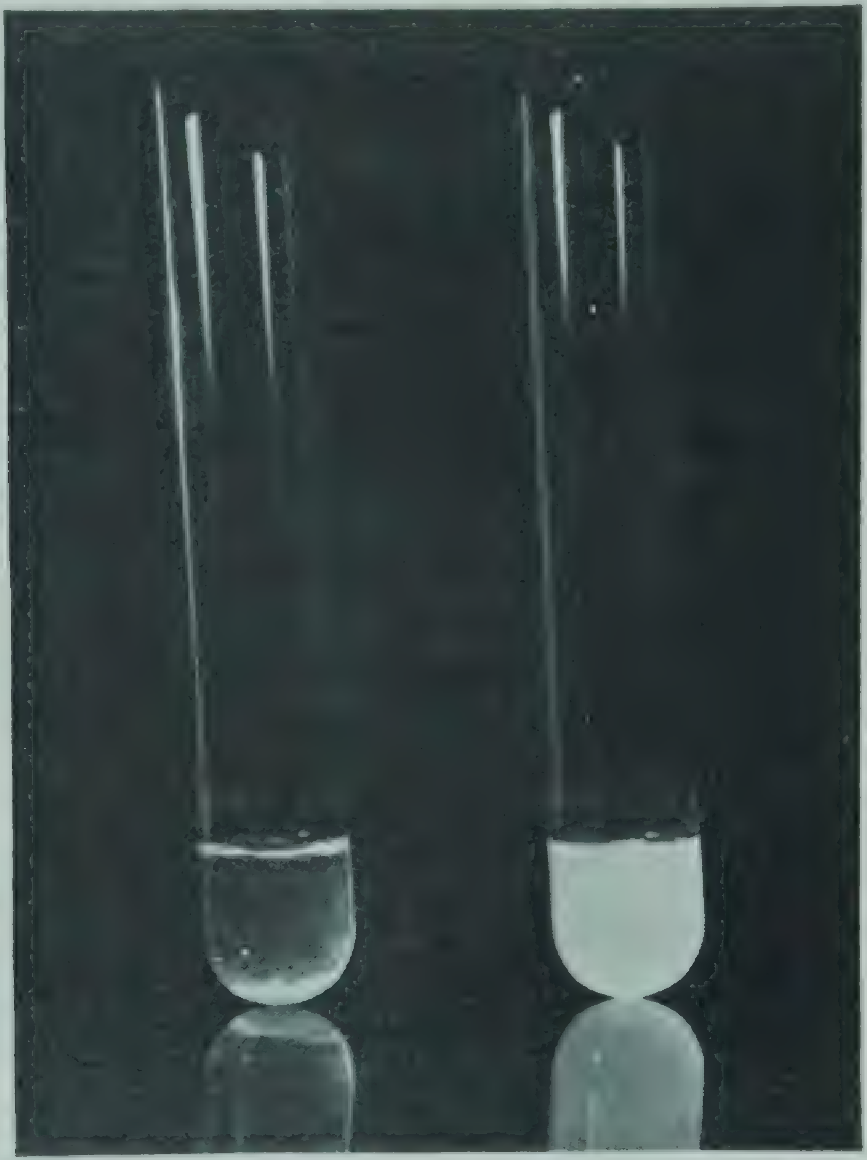


Fig. 2: Cephalin-cholesterol-flocculation test.
Left: positive. *Right*: negative.

Hanger considered it a means of differentiating obstructive jaundice from parenchymatous jaundice. The results of the flocculation test in patients with obstructive jaundice, however, depend on the amount of associated liver damage; and this in turn depends on the degree of obstruction, duration of obstruction, and presence of associated infection.

Moore and co-workers[6] have advanced the hypothesis that positive flocculation may result from any of the following alterations in serum: (1) the presence of excess amounts of gamma globulin in such quantities that there are insufficient normal components of the serum-albumin fraction to inhibit the reaction; (2) a decrease in the serum-albumin fraction below levels necessary to inhibit the reaction; and (3) a diminution in the flocculation-inhibiting properties of the albumin fraction, as demonstrated in cases of hepatitis. The positive result observed in hepatitis is thought to be due to a combination of all factors, especially a modification of the albumin fraction.

The test is negative in normal persons and negative or weakly positive (1+) in patients with obstructive jaundice. It is strongly positive in hepatitis, catarrhal jaundice, and cirrhosis.

The cephalin-cholesterol-flocculation test has some prognostic value in acute hepatitis. The progressive decrease and final disappearance of an initial, strongly positive reaction indicates a good prognosis, whereas the persistence of a strongly positive reaction indicates liver degeneration and a poor prognosis.

According to Hanger,[6] the cephalin-cholesterol-flocculation test is not specific for liver disease. It may be positive in nephrosis, bacterial endocarditis, virus and pneumococcus pneumonia, disseminated lupus erythematosis, infectious mononucleosis, and terminal nephritis.

Thymol-Turbidity Test:

According to Maclagan,[7] turbidity is produced in a barbital buffer saturated with thymol when serum from patients with liver disease is added to it.

The degree of turbidity apparently parallels the degree of liver damage. An analysis of the precipitate demonstrated that it contained globulin, phospholipid, cholesterol, and thymol. The mechanism is unexplained by Shank and Hoagland,[8] but they believe there is some evidence to indicate that the reaction may be due to an abnormal globulin released into the bloodstream in the presence of liver damage.

The normal range is from 0 to 4 Maclagan units; and in patients with parenchymatous liver disease, the values are increased. Shank and Hoagland[8] found the values increased in eighty-two out of eighty-three patients with infectious hepatitis, the mean level being 17.03 units. During convalescence, there was a gradual decrease from the early high values to normal. Increased values were also noted in cases of toxic hepatitis and cirrhosis.

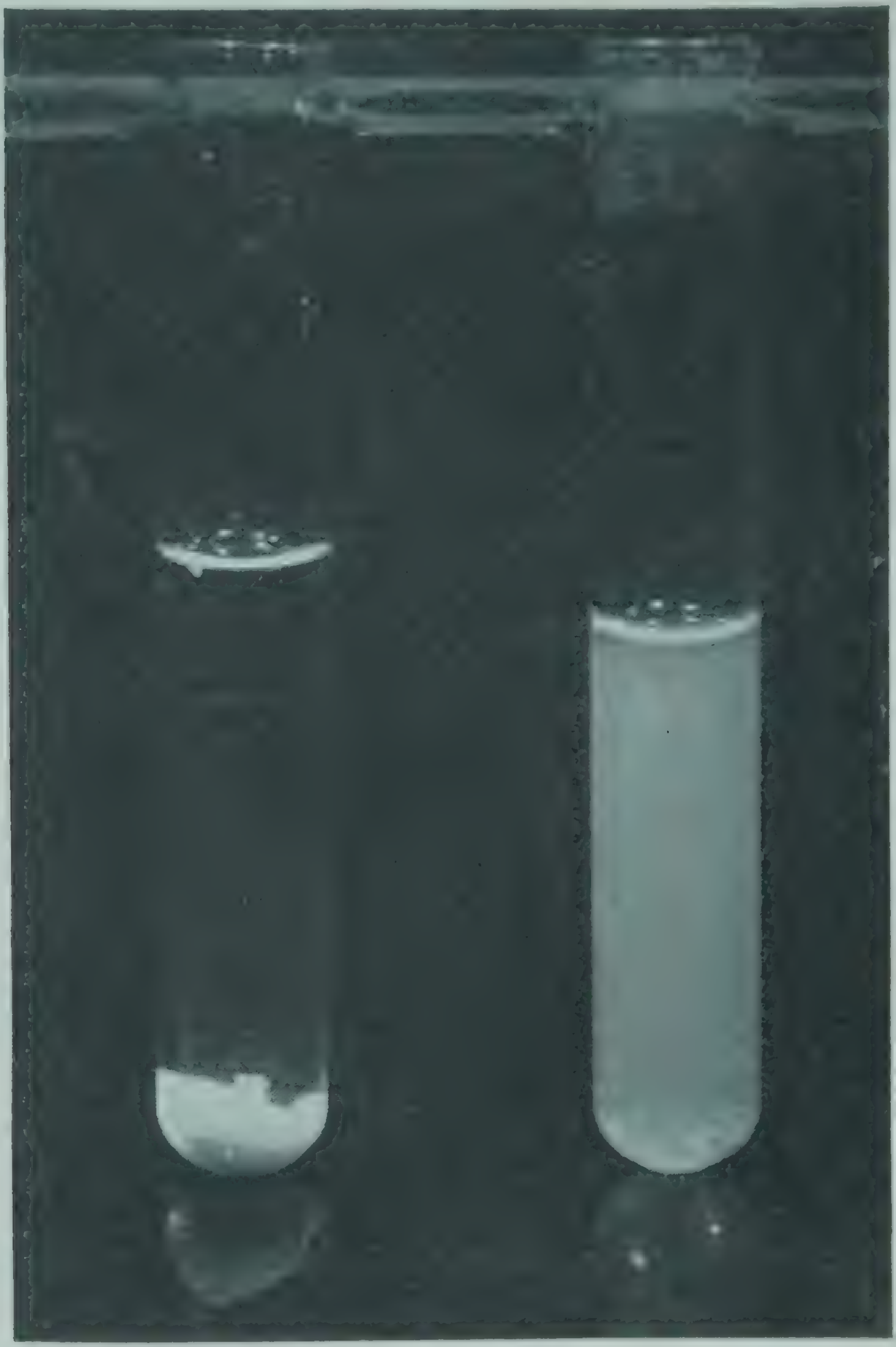


Fig. 3: Thymol-flocculation test. *Left:* positive. *Right:* negative.

Thymol-Flocculation Test:

The degree of flocculation is read after eighteen hours from the tube set up for the thymol-turbidity test. Like the cephalin-cholesterol-flocculation test, it is expressed as 1+ through 4+ depending on the amount of flocculation.

Degrees greater than 1+ are considered abnormal. In a series of twenty-eight cases of infectious hepatitis studied by Neefe and Reinhold,[9] the flocculation test was positive in all cases some time during the disease. The flocculation test became positive in 68 per cent of the cases before the thymol-turbidity test and in a few cases before the onset of symptoms.

In the same series, the cephalin-flocculation test also becomes positive in all cases some time during the disease. The cephalin-flocculation test became positive before the thymol-flocculation test in 47 per cent of the cases, while the reverse was true in 21 per cent. Both became positive at the same time in the remaining 32 per cent of the cases.

The thymol-flocculation test is not recommended as a substitute for the turbidity test because the latter when positive gives a quantitative estimate of the abnormality in thirty minutes.

Takata-Ara Test:

This test is based upon the assumption that mercuric chloride and sodium carbonate form mercuric oxide in the presence of proteins.

In certain pathological conditions, precipitation of protein (especially globulin) results. The test is usually negative in normal persons. Unfortunately, the test is not specific for liver disease. It is positive in portal cirrhosis, severe hepatocellular jaundice, and liver abscess, but it may be positive also in chronic passive congestion of the liver, carcinoma of the liver, pulmonary tuberculosis, hyperthyroidism, multiple myeloma, nephritis with edema, and chronic alcoholism. It is generally negative in syphilis involving the liver, cholelithiasis without obstruction, and catarrhal jaundice.

Icterus Index:

An increase of bilirubin in the serum gives it a color ranging from deep yellow to amber. The depth of the color is determined arbitrarily by comparing the serum with a standard of potassium-dichromate solution.

The color change may be the result of obstructive or hemolytic jaundice. The icterus index does not aid in differentiating prehepatic from posthepatic bilirubin.

van den Bergh Reaction:

The van den Bergh reaction is helpful in differentiating obstructive jaundice from hemolytic jaundice.

There are two main types of reactions, the direct and indirect. The direct reaction takes place without the addition of alcohol, while the indirect requires the addition of alcohol both to the serum and Ehrlich's diazo reagent.

The serum in a patient with obstructive jaundice gives a prompt, direct reaction. The serum in a patient with hemolytic jaundice and normal serum give only the indirect reaction.

Quantitative-Bilirubin Test:

The quantitative determination of bilirubin is preferred to the icterus index.

The indirect van den Bergh reaction is used as the basis for the quantitative determination of bilirubin in all types of sera. After the color reaction has developed, the serum is compared in a colorimeter with a standard made from anhydrous cobaltous sufate.

The normal range is from 0.2 to 1.0 mg. per 100 cc. Increased bilirubin values are found both in obstructive and hemolytic jaundice. The test is of value in detecting latent jaundice and in following the progress of a case of obvious jaundice. A total serum bilirubin of from 10 to 12 mg. per 100 cc. is the equivalent of an icterus index of 100.

Alkaline-Phosphatase Test:

As previously stated, the normal range of alkaline-serum phosphatase for adults is from 1.5 to 4 Bodansky units per 100 cc. Roberts[10] first advanced the view that the estimation of the alkaline phosphatase might serve as a diagnostic aid in cases of jaundice. In biliary obstruction, there is a greater increase in phosphatase than there is in hepatitis. Values below 10 Bodansky units are said to indicate hepatic jaundice, and values above this level are said to indicate extrahepatic, obstructive jaundice.

Cantarow and Nelson,[11] however, reported broad overlapping of values in hepatocellular and obstructive jaundice, and concluded that serum phosphatase had no value in differentiating these types of jaundice. Gutman and co-workers[12] concluded that the test is most valuable in excluding, with a high degree of probability, obstruction of the common duct in patients with no increase of serum phosphatase.

Plasma Proteins:

Since the liver plays a major role in the production of albumin and globulin, it is reasonable to expect a reduction of these compounds in liver disease. In parenchymatous liver disease (hepatocellular hepatitis, acute yellow atrophy, and toxic hepatitis) and in cirrhosis, the total plasma proteins, particularly the albumin fraction, are decreased.

Free and Total Cholesterol:

According to Sperry,[13] there is a wide range in the normal level of total cholesterol in the blood, but the ratio of free to total cholesterol varies within narrow limits. Jones[14] used heparinized plasma in a series of normal persons and reported the following values:

	Mg. per 100 Cc.
Cholesterol ester	132 (±21)
Free cholesterol	57 (±10)
Total cholesterol	189 (±21)
Proportion of free in total cholesterol	30 (± 2)

An increase in the proportion of free in total cholesterol above 30 per cent is usual in infectious hepatitis. Hoagland and Shank[15] found the ratio of free to total cholesterol to exceed 30 per cent in 140 of 163 patients with infectious hepatitis.

Jones[14] and others have found that obstruction of the bile ducts usually produces hypercholesterolemia, while primary liver disease usually leads to decreased plasma levels. Thannhauser and Schaber[16] correlated reduction of cholesterol ester with liver damage and found practically complete disappearance of the ester in patients with acute yellow atrophy.

Epstein and Greenspan[17] reported a total, blood-cholesterol level of more than 300 mg. per 100 cc. in 82 of 105 patients with obstructive jaundice and an increase in cholesterol ester above 150 mg. per

100 cc. in 79 patients of this series. Patients with bile-duct obstruction due to carcinoma had higher, total, cholesterol values than those with obstruction due to a calculus.

Glucose Level:

The fasting, glucose, blood level should not be considered of primary importance in the diagnosis of liver damage. In marked hepatocellular damage, the fasting, glucose, blood level is decreased because the liver is unable to store a reserve of glycogen. This is illustrated by a case of complete fatty degeneration of the liver reported by Goodale.[18] This patient had a glucose level of 25 mg. per 100 cc. on admission and died shortly thereafter.

Colloidal-Gold Test:

The basis of the colloidal-gold test is the precipitation of colloidal gold by serum. It is very sensitive and consequently gives false-positive reactions.

In normal persons, the first tube is not higher than 4, with less precipitation in the remaining tubes. In patients with liver damage, there is complete precipitation in the first tube and in one or two more, while the remaining tubes show less precipitation.

If circumstances do not permit the use of both tests (colloidal-gold, and thymol-turbidity and flocculation), it appears that the thymol-turbidity and flocculation test is more informative than the colloidal-gold test.[9]

Vitamin-A Level of Plasma:

Since the liver is the main storage place for vitamin A, liver diseases may be expected to interfere with the metabolism of this vitamin. In fact, reduced vitamin-A levels (see p. 535) in the liver and blood plasma, and defective dark adaptation have been reported to result from deficiency of vitamin A due to liver diseases.

Leitner and Moore[19] reported a mean level of plasma-vitamin A as 118 International units per 100 cc. for a group of forty-one healthy adults. Harris and Moore[20] found that the mean level of plasma-vitamin A was 54.6 I. U. in the first four days of infective hepatitis. Successive estimations showed a continuous rise until a mean level of 114.3 I. U. was reached. Plasma-carotene blood levels were also low early in the disease, the mean level for the first five days being 67.2 I. U. as compared with 161 I. U. for healthy adults. During recovery, the mean level rose to 118 I. U. for from twenty to twenty-nine days after admission.

Meyer and co-workers[21] found the lowest averages of plasma-vitamin A in progressive cirrhosis with superimposed hepatitis and manifest jaundice. They also state that a normal blood level of vitamin A does not necessarily exclude some damage to the liver, and a zero, or close-to-zero, level is definite evidence of damage to the liver provided nutritional factors can be excluded.

CHANGES IN URINE AND FECES SECONDARY TO LIVER DAMAGE AND BILIARY OBSTRUCTION

Urobilinogen Test:

Urobilinogen is formed in the intestine by the action of bacteria on bilirubin, which is excreted by the liver. The amount of urobilinogen in the feces depends on the quantity of bilirubin reaching the intestine, while the amount of urobilinogen in the urine is influenced by the ability or lack of ability of the liver to reexcrete it into the bile ducts. If the liver has sustained hepatocellular damage, it is less able to reexcrete urobilinogen into the bile, and consequently there will be a greater amount in the urine.

Under certain conditions, there may be little urobilinogen returning to the liver by way of the portal system, leaving little or none to be reexcreted by the liver or to escape into the general circulation to be excreted by the kidneys. These conditions are as follows: (1) diarrhea or any other cause of malabsorption from the colon; (2) decreased formation of bilirubin because of diminished rate of blood destruction, as seen in certain anemias; and (3) complete obstruction of the outflow of bilirubin into the intestine, allowing little or no opportunity for formation of urobilinogen.

Watson[22] gives the usual values of urobilinogen in the feces as from 100 to 200 mg. per day (Table 1). The normal values for urobilinogen in the urine are from 0.5 to 1.5 mg. per day.

The fecal urobilinogen is greatly increased in hemolytic jaundice because of the increased production of bilirubin, while that in the

TABLE 1: UROBILINOGEN EXCRETION IN JAUNDICE*

Type of Jaundice	*Feces, Mg. per Day*	*Urine, Mg. per Day*
None	100 to 200 (usual range)	0.5 to 1.5 (usual range)
Hemolytic	600 to 2000 (usual range)	5 to 30 (usual range)
Neoplastic	5.0 (90 per cent of cases)	0.5 (90 per cent of cases)
Calculous	0 to 300	0 to 50
Parenchymal	10 to 300 (usual range)	0.5 to 100 (usual range)

* Schiff, L.: Differential Diagnosis of Jaundice. The Year Book Publishers, Inc., Chicago, 1946, p. 234. (Based on data from Watson, C. J.: The Bile Pigments. New England J. Med., 227:705, 1942.)

urine is only moderately increased. The latter is thought to be the result of subnormal hepatic formation rather than of increased urobilinogen formation in the intestine.[22,23]

In jaundice due to obstruction by calculus, the amount of urobilinogen in the urine depends upon certain factors: (1) the duration of the obstruction and the resulting liver damage, (2) the extent of obstruction, and (3) the presence or absence of cholangitis. Biliary obstruction is incomplete in 90 per cent of obstructions by calculi; and in such cases, the urobilinogen in the urine may be normal or decreased. With complete biliary obstruction, the amount of urobilinogen both in the urine and feces is markedly decreased.

According to Watson,[22] there is complete obstruction in 90 per cent of the cases of obstruction caused by neoplasm. He found a daily, fecal, urobilinogen content of less than 5 mg. and a daily urinary level of less than 0.5 mg.

In acute hepatitis (parenchymal jaundice), the fecal urobilinogen may or may not be decreased, but the urinary urobilinogen is considerably increased because of the decreased ability of the liver to reexcrete into the bile the urobilinogen returned to it from the intestine. Sometimes at the height of the jaundice, bile may not enter the intestine, and the fecal and urinary, urobilinogen, blood levels may drop in proportion.

According to Watson,[22] if simple inspection or a qualitative test of feces is carried out, it appears that complete exclusion of bile is more common than it actually is. He found that grossly acholic stools may contain from 10 to 15 mg. of urobilinogen per day, and a twenty-four-hour sample of urine may contain 15 mg. of urobilinogen.

When acute hepatitis is regressing, urobilinogen usually appears in the urine in fairly large amounts because of the residual liver damage. During this time, urobilinogen in the feces may or may not be increased.

Bilirubin Test:

Bilirubin is increased in the urine both in obstructive jaundice and hemolytic jaundice. The posthepatic bilirubin, which gives a direct van den Bergh reaction, is more diffusible than the prehepatic bilirubin, which gives an indirect reaction. Consequently, the bilirubin of obstructive jaundice is seen earlier in the urine. In fact, bilirubin may be absent in the urine in hemolytic jaundice with a fairly high level of bilirubin in the blood.

Tyrosinuria Test:

The urine contains characteristic crystals of leucine and tyrosine as the result of conditions in which there is extensive fatty degeneration, for example acute yellow atrophy, eclampsia, and poisoning by chloroform, phosphorus, arsphenamine, and carbon tetrachloride. Tyrosinuria is not seen in frank infections and obstructions of the bile ducts but may occur in conditions associated with extensive necrosis, such as degenerating bronchogenic carcinoma.

LIVER BIOPSY

Recently, there has been renewed interest in liver biopsies, as evidenced by the number of reports on the subject. They have added a great deal to our knowledge of the stages of acute and chronic hepatitis as well as other liver conditions.

A liver biopsy may be obtained directly during a laparotomy, during a peritoneoscopy, or by the transpleural aspiration method. In carrying out the biopsy, it is advisable to choose cooperative patients and eliminate those who have a tendency to bleed.

Sherlock[24] reported 8 deaths as a result of liver biopsy in 1200 cases, but she believes this figure can be reduced by using her modified technic and by the selection of cases.

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CHAPTER 13

Bacteriological Examinations

INTRODUCTION

Bacteriological examination of material or body fluids is of great importance to the clinician because frequently the causative agent of an infection is found in the stained smear or culture. This is usually true of the findings in such fluids as blood, pleural exudate, and cerebrospinal fluid. In the reports of throat, sputum, and stool cultures, however, the clinician must correlate the findings with the condition of the patient. Furthermore, he must evaluate a reported organism, as to whether it is causing the current illness or indicates a carrier state. The typhoid bacillus reported in a stool examination may indicate active typhoid fever, or it may be only an incidental finding in a carrier. In addition to identifying an organism, it is sometimes desirable to determine its virulence. This is important in diphtheria and gas-bacillus infections.

Sometimes the causative organism is not found, as in the case of brucellosis. The presence of specific agglutinins with increasing titers is helpful, however, in making the diagnosis in such cases.

CHEMOTHERAPEUTIC DRUGS IN RELATION TO BACTERIOLOGY

Bacteriological examinations have assumed new importance since the advent of chemotherapeutic drugs and antibiotics. Until the discovery of the sulfonamides, chemotherapy was limited to arsenicals, antimony, bismuth, quinine, quinoline, and certain dyes, such as trypan red and the flavines. These agents can be used only for a limited number of infections, such as those caused by certain spirochetes and protozoa. Pathogenic bacteria are resistant to these chemical agents.

The discovery of the efficacy of the sulfonamide compounds in treating bacterial infections initiated a new era in chemotherapy. The different sulfonamides have various solubilities, degrees of toxicity, rates of absorption and excretion, and variations in therapeutic efficacy.[1] The sulfonamides are bacteriostatic rather than bactericidal and apparently function in vivo by suppressing the multiplication of bacteria. This gives the normal, body-defense mechanisms, chiefly phagocytosis, an opportunity to function. According to Woods,[2] the bacteriostatic action of sulfanilamide is neutralized by *para*-aminobenzoic acid, which is required as a growth accessory substance by most bacteria. Bacteria appear, however, to be able to synthesize *para*-aminobenzoic acid so that it is not necessary to have it supplied. Woods[2] and Fildes[3] formulated the theory that the action of the sulfonamides on bacteria is the result of a competition of these compounds (sulfonamides) with an essential bacterial metabolite, *para*-aminobenzoic acid. Since *para*-aminobenzoic acid plays such an important part in the metabolism of bacteria, it seems reasonable to assume that any agent that neutralizes it would thus inhibit the growth of such organisms.

It should be remembered that any body fluids from a person receiving adequate sulfonamide therapy may contain enough of the chemical to inhibit bacterial growth. This inhibiting effect may be eliminated by adding 5 mg. per 100 cc. of *para*-aminobenzoic acid to the culture medium.

ANTIBIOTIC DRUGS IN RELATION TO BACTERIOLOGY

Substances formed by certain bacteria and fungi are toxic for some bacteria, chiefly gram-positive organisms. Such substances have been called "antibiotics" by Waksman.[4] These substances include polypeptides, sulfur-containing compounds, lipids, pigments, quinones, and organic bases. Some of these agents have been prepared in pure crystalline form, and in some cases the structure is known. The antibiotics have become very important as therapeutic agents because certain of them are very effective in destroying organisms, yet have minimal toxicity for human beings.

Antibiotic Agents from Bacteria:

According to Jordan and Burrows,[5] the best-known antibiotics of bacterial origin are produced by Pseudomonas aeruginosa. Two substances are formed: (1) a chloroform-soluble, phenazine compound, pyocyanin; and (2) pyocyanase, a substance that appears to be a lipid, whose activity is associated with the presence of unsaturated fatty acids in the molecule. Another substance, hemipyocyanin, which is a derivative of pyocyanin, may also be isolated by ether extraction. Unfortunately, these agents are toxic for man.[6,7]

Dubos[8] succeeded in isolating an alcohol- and water-soluble, polypeptide antibiotic from Bacillus brevis. He called this substance "tyrothricin."

Antibiotic Agents from Molds:

The best known antibiotic from molds is penicillin, which is derived from Penicillium notatum. Penicillin, fortunately, has the properties of effective bacteriostasis and low toxicity for man. It was discovered by Fleming[9] in 1929, but it was not until 1940 that its possibilities as a chemotherapeutic agent were discovered by Chain and his associates.[10] Penicillin is soluble in water, can be extracted from an acidified water solution with ether or amyl acetate, and can then be extracted from this solvent by shaking with dilute barium hydroxide or sodium-bicarbonate solution. The barium and sodium salts are stable.

When bacteria are exposed to penicillin in vitro, they become swollen and do not divide. For some reason, penicillin is most effective in combating infections by gram-positive organisms, but the mechanism by which it inhibits growth is not known.

Practical experience with penicillin in the armed forces during the second World War, and in civilian practice since the war, has confirmed the fact that it has low toxicity for man and is a very effective therapeutic agent in vivo. In addition to its efficacy in the treatment of infections caused by gram-positive bacteria, it appears to offer hope for the treatment of primary syphilis with a relatively short series of inoculations. Primary lesions are readily cleared up, but the ability of penicillin to maintain negative serology is still under investigation.

Body fluids from persons under treatment with penicillin may contain some of the agent, which would inhibit the growth of the organisms in vivo. It is important, therefore, to add an agent, penicillinase, to the media to neutralize any penicillin present in the fluid under culture.

Antibiotic Agents from Actinomycetes:

Waksman and his associates[11,12] isolated a number of agents from Actinomycetes. The Actinomycetes are considered to be neither bacteria nor fungi, in fact many consider them as transition forms between these two forms of life. The most effective agent isolated from Actinomycetes by Waksman[13] is streptomycin, which he found in cultures of Actinomyces griseus. Streptomycin has a low toxicity for man and is effective in the treatment of infections caused by gram-negative bacteria. Thus, it supplements penicillin, which is more effective in treating infections caused by gram-positive organisms. In addition to the above-mentioned discoveries, recent work in Veterans Administration tuberculosis sanatoria[14] indicates that streptomycin is effective in suppressing the growth of the Mycobacterium tuberculosis in vivo. It is also used to prepare patients with pulmonary tuberculosis for thoracoplasty and lobectomy.

Thus far, no agent has been publicized that will neutralize streptomycin in culture media.

RESISTANCE OF ORGANISMS

Certain organisms develop resistance to chemotherapeutic agents, while others are naturally resistant. For example, gram-negative bacilli are more resistant to penicillin than to streptomycin. Whether an organism can become drug-resistant in vivo and be transmitted to the population while remaining resistant is a question of practical importance.

With the general use of the sulfonamide drugs in the treatment of gonorrhea, an increasing number of cases with sulfonamide-fast gonococci have been encountered. It has been suggested that this increase may be due to the development of sulfonamide-fastness in vivo, possibly, in part, through inadequate self-medication or an increased prevalence of sulfonamide-fast strains arising as a result of eliminating susceptible strains of therapy.

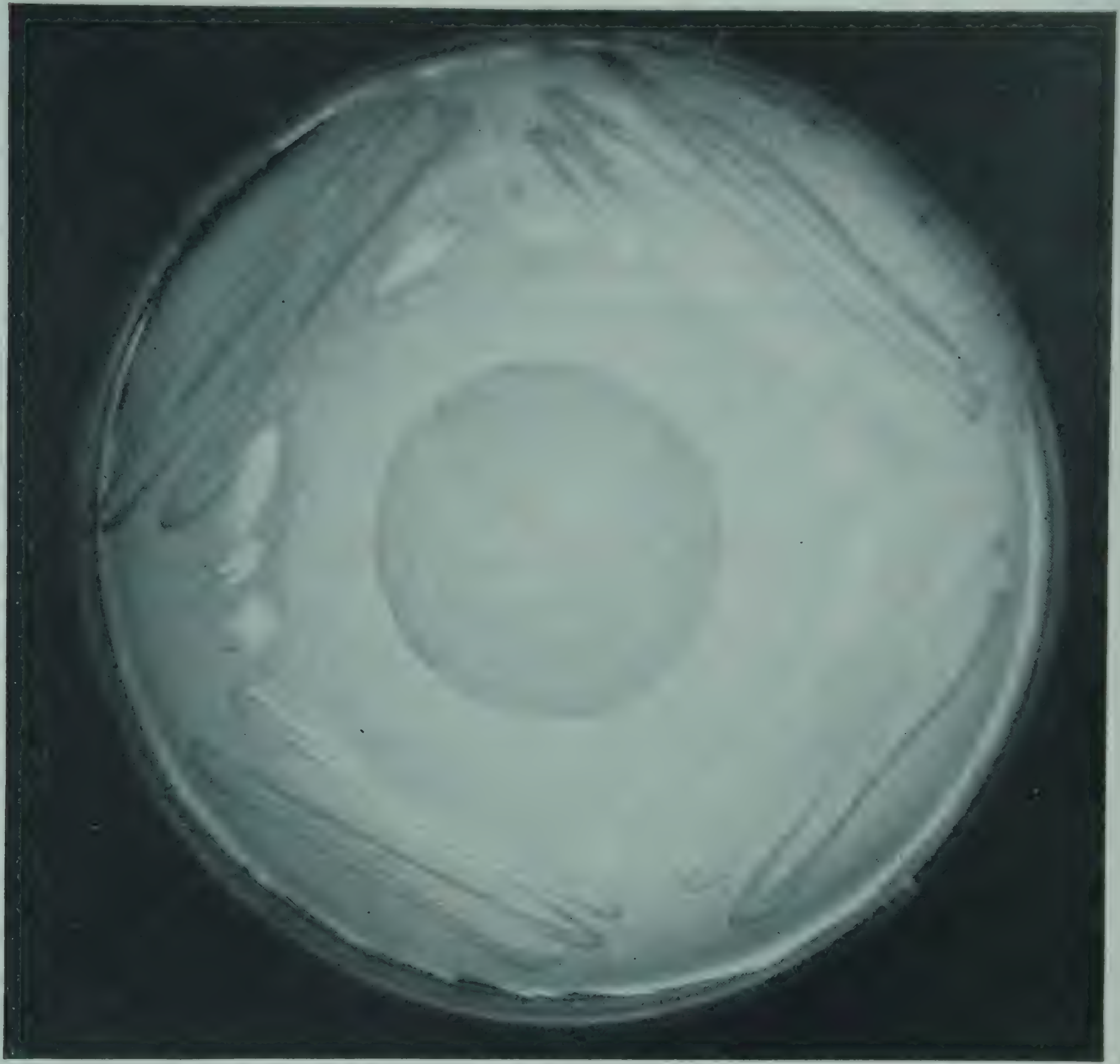

Fig. 1: Effect of penicillin on Staphylococcus aureus. Note absence of growth around filter paper, which is saturated with penicillin.

Some organisms have developed resistance to penicillin and others to streptomycin. Before embarking on a course of therapy, which may be expensive, it is sometimes advisable to test the susceptibility of the organism in vitro to one or both of the above-mentioned antibiotics. This may be done qualitatively by saturating a round piece of filter paper with the drug and placing it on media in a Petri dish that has been planted with the organism. If the organism does not grow immediately around the paper, the organism is susceptible to the drug.

The susceptibility of organisms may be quantitatively determined by making serial dilutions of the drug in liquid media and inoculating it with the organism. The tube with the highest dilution in which there is no growth indicates the efficacy of the drug. This is of value only when compared with the efficacy of the drug during the course of treatment, to see if the organism develops increasing resistance.

BLOOD

Blood Cultures:

Blood culture is a common means of determining the organism that causes an infection. The reliability of the report, however, depends on technic because contaminating organisms are readily picked up when the operator is careless in inoculating the medium. Such organisms as Escherichia coli, Bacillus subtilis, Corynebacterium diphtheriae, and Staphylococcus albus are the most common contaminants.

It is advisable to supplement aerobic methods with anaerobic procedures because certain streptococci and Clostridium perfringens are anaerobes. The nature of the treatment, if instituted, should be ascertained so that either *para*-aminobenzoic acid or penicillinase, or both, can be added to the medium.

Evaluation of Nature of Infection:

Following the report of an organism in a blood culture, it is necessary for the clinician to evaluate the nature of the infection. The organism may be the result of either a bacteremia or septicemia.

Bacteremia: A bacteremia is a transient invasion of the blood by bacteria with no clinical evidence of infection. Such an invasion occurs after a meal if the patient has apical tooth abscesses, or after extraction of teeth under general or local anesthesia. Bacteremia has been shown to occur in the following instances: (1) by Murray and Moosnick[15] in persons with infection of teeth after chewing, (2) by Okell and Elliott[16] in from 60 to 70 per cent of patients after extraction of infected teeth under general anesthesia, (3) by Burket and Burn[17] in approximately 17 per cent of patients after extraction of teeth under local anesthesia, and (4) by Southworth and Flake[18] before and after removing infected tonsils. In fact, the manipulation of any infected tissue is a potential source of bacteremic organisms.

Septicemia: Septicemia is invasion of the blood by micro-organisms that multiply, produce toxins, and cause clinical evidence of infection. Septicemia may occur as a result of infection of fixed tissues, for example pneumonia, or septicemia may cause infection of fixed tissues in the form of septic embolic infarcts.

The organisms that commonly cause septicemia are the following:

Streptococci
Staphylococci
Pneumococci
Meningococci
Eberthella typhosa
Brucella abortus
Brucella melitensis

Less common causes of septicemia are as follows:

Escherichia coli
Bacillus anthracis
Mycobacterium tuberculosis
Gonococci
Pseudomonas aeruginosa
Clostridium tetani
Clostridium perfringens
Hemophilus influenzae
Klebsiella pneumoniae

Although not usually considered in the category of septicemia, certain spirochetes occur in the bloodstream. Unfortunately, they do not lend themselves to ordinary cultural methods. The Leptospira icterohaemorrhagiae, which causes spirochetal jaundice (Weil's disease), may be identified preferably by inoculating guinea pigs with blood and urine. The Treponema pallidum, which causes syphilis, and Treponema pertenue, which causes yaws, occur in the bloodstream and can be identified by inoculating the testicle of a rabbit. This procedure is not necessary, however, because darkfield examination of primary lesions and flocculation tests are simpler methods of making a diagnosis. In relapsing fever and rat-bite fever, the spirochetes are preferably identified by examination of stained smears of the blood. In fusospirochetosis, the fusiform bacilli and spirochetes can be cultured from the blood on rare occasions, and then only when they are grown under completely anaerobic conditions.

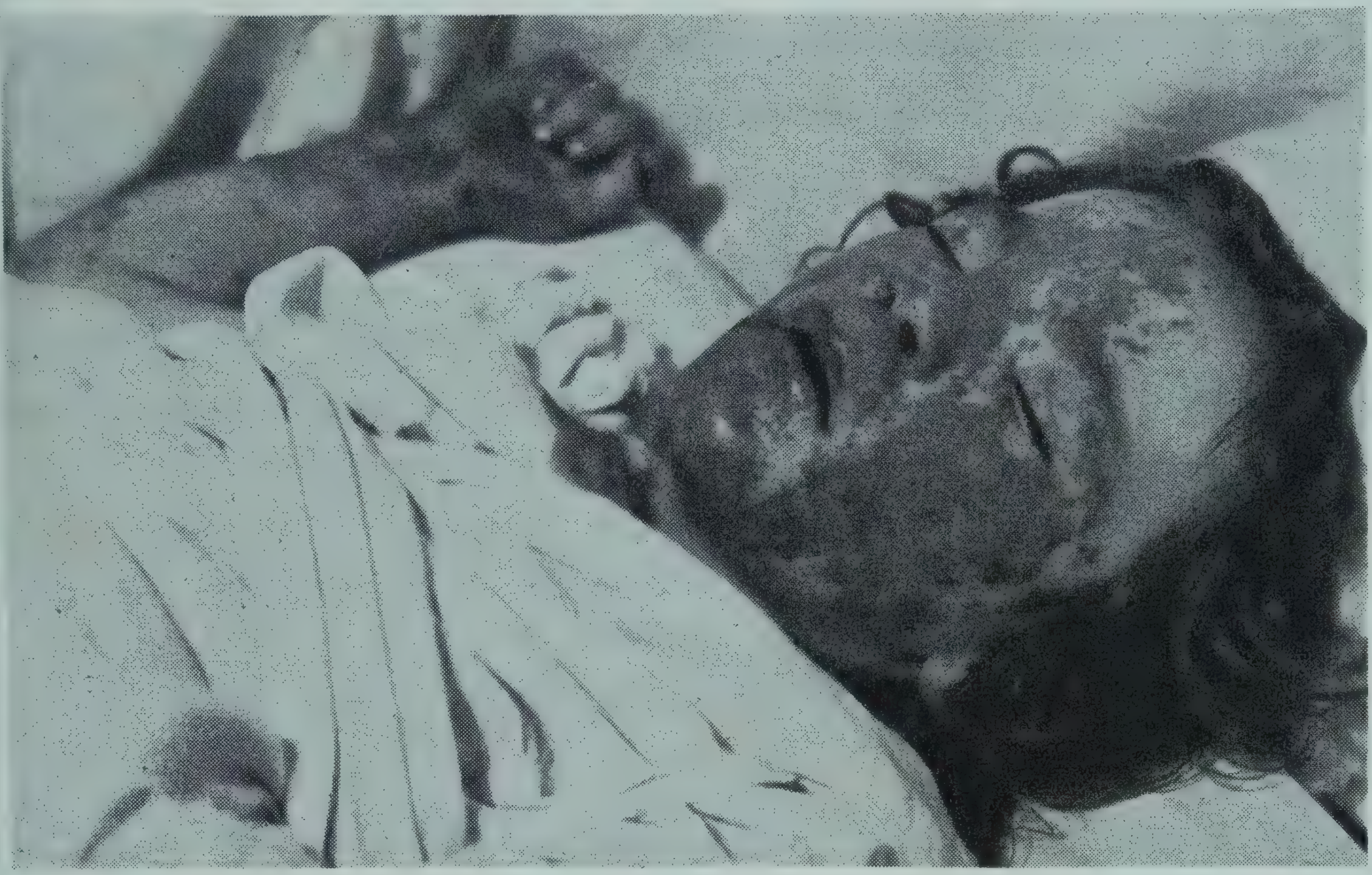

Fig. 2: Meningococcemia with purpura.

URINE

Bacteriological examination of the urine is of great importance to the urologist in determining the causative organisms of cystitis, pyelitis, and pyelonephritis. The identification of the Mycobacterium tuberculosis from a single kidney is the final word in the diagnosis of tuberculosis of the kidney. In typhoid fever, the Eberthella typhosa is sometimes cultured from the urine, but the diagnosis is usually made before this time.

Collection of Specimens:

It is of the utmost importance that specimens be collected from the kidneys and bladder without introducing contaminating organisms. This means that the parts must be thoroughly cleaned with soap and then sterilized with an antiseptic, such as merthiolate. The kidney or bladder catheters must be sterile, and the containers for the specimens must also be sterile. After collecting the specimens, the containers must be protected with sterile covers so that organisms cannot enter from the air.

When an organism is reported, especially a staphylococcus or Escherichia coli, it must be evaluated skeptically because of the possibility of having introduced such contaminants from the meatus of the urethra.

Bacteriological Findings:

In Pyelonephritis: The organisms most commonly found in the urine in pyelonephritis are Escherichia coli, Proteus vulgaris, staphylococci, streptococci, Eberthella typhosa, and Mycobacterium tuberculosis. Escherichia coli, staphylococci, streptococci, and Proteus vulgaris may ascend from the bladder to one or both kidneys. On occasion, staphylococci and streptococci may lodge in the kidneys in the course of septicemia.

The finding of Eberthella typhosa in the urine occurs after the second week of the disease (in from 20 to 30 per cent of the cases) and in a very small number of recovered patients who are carriers. The Mycobacterium tuberculosis is found in the urine in tuberculosis of the kidneys, bladder, or prostate when the lesion has broken down and empties into the urinary tract. Such lesions are secondary to tuberculosis elsewhere in the body, usually in the lungs. When acid-fast bacilli are found in catheterized specimens of urine, they are likely to be Mycobacteria tuberculoses. Contamination with the Mycobacterium smegmatis is extremely unlikely under these conditions. The pathogenicity of the acid-fast bacillus is determined by inoculation of a guinea pig.

Other systemic diseases in which the causative organisms may be found in the urine are dysentery (Shigella dysenteriae), undulant fever (Brucella abortus and Brucella melitensis), and spirochetal jaundice (Leptospira icterohaemorrhagiae).

In Cystitis: The organisms most commonly found in primary infection of the bladder are Escherichia coli, staphylococci, streptococci, and Proteus vulgaris. Cystitis rarely is caused by gonococci and pneumococci. Pseudomonas aeruginosa, Neisseria catarrhalis, Gaffkya tetragena, and Aerobacter aerogens may be cultured from the bladder but are not considered pathogenic.

GASTRIC AND DUODENAL CONTENTS

Gastric Contents

Normal Flora:

The stomach receives organisms that are swallowed in the saliva, food, and drink. Many of these organisms are killed by the hydrochloric acid of the stomach when it is present in normal amounts. According to Bartle and Harkins,[19] aspirated gastric fluid with from 60 to 100 degrees of free hydrochloric acid killed all bacteria tested in vitro, with the exception of Lactobacillus acidophilus. Gastric juice with from 20 to 40 degrees of free hydrochloric acid killed streptococci and Escherichia coli in vitro but did not kill staphylococci or Lactobacillus acidophilus. Low hydrochloric-acid content of from 0 to 5 degrees had no demonstrable bactericidal effect. Spore-bearing bacilli can apparently resist the action of hydrochloric acid.

In the normal stomach, the organisms that are usually found are Lactobacillus acidophilus, staphylococci, Bacillus subtilis, fungi, and yeasts.

Significance of Other Bacteria:

Lactobacilli, particularly Lactobacilli of Boas-Oppler, are found in cases of obstruction with stasis of gastric contents, as in carcinoma of the stomach. The finding of these Lactobacilli, however, is not diagnostic of carcinoma. In examining for these organisms, it is preferable to examine a smear immediately stained by Gram's method.

Mycobacteria tuberculoses are swallowed by persons who have these organisms in the secretions from the lungs. In some cases with negative sputum, the gastric contents will show Mycobacteria tuberculoses, particularly in a guinea pig. It is preferable to inoculate a guinea pig because other, nonpathogenic, acid-fast bacilli may be present in the stomach and will show up in smear and culture.

DUODENAL CONTENTS

Normal Flora:

If bacteria escape from the stomach without being killed, they find a more favorable media for growth in the duodenum. Consequently, there is a greater variety of bacteria, which include not only those of the stomach but also streptococci, Escherichia coli, and sometimes Clostridium perfringens and bacterioids.

Significance of Other Bacteria:

Examination of the duodenal contents is not usually made because the inconvenience to the patient is greater than the value of information received as to infection of the duodenum proper or the gallbladder. Bacillus anthracis may be found in the duodenal contents where there is infection elsewhere in the intestinal tract.

Cholecystitis, cholangitis, and hepatitis may be present, caused by bacterial infection, but the bacteria are not always found in the bile. Causative organisms of these infections, such as streptococci, staphylococci, and Escherichia coli, have been isolated from the duodenal contents, however. The Leptospira icterohaemorrhagiae, which causes spirochetal jaundice, may also be found in the bile in the duodenum.

CEREBROSPINAL FLUID

Normal:

Normal cerebrospinal fluid is sterile. It must be collected under aseptic conditions in order to prevent contamination. If bacteria, particularly staphylococci, do grow on culture, they should be correlated with the clinical condition, cell count, total protein, and glucose, spinal-fluid level. Unless changes are found in the examinations, these organisms are probably contaminants.

The fluid is also bacteriologically sterile in lymphocytic choriomeningitis, serous meningitis, and chemical meningitis following the intrathecal injection of sterile agents.

In Purulent Meningitis:

Acute purulent meningitis may be caused by meningococci, Hemophilus influenzae, pneumococci, hemolytic streptococci, staphylococci, and occasionally Escherichia coli, Eberthella typhosa, and Mycobacterium tuberculosis. A smear by Gram's method from the sediment or pellicle should be prepared as the first step in the diagnosis. When an organism is found, it should be reported to the doctor immediately so that appropriate treatment may be instituted. A culture should be made immediately so that complete identification of the organism may be made ultimately.

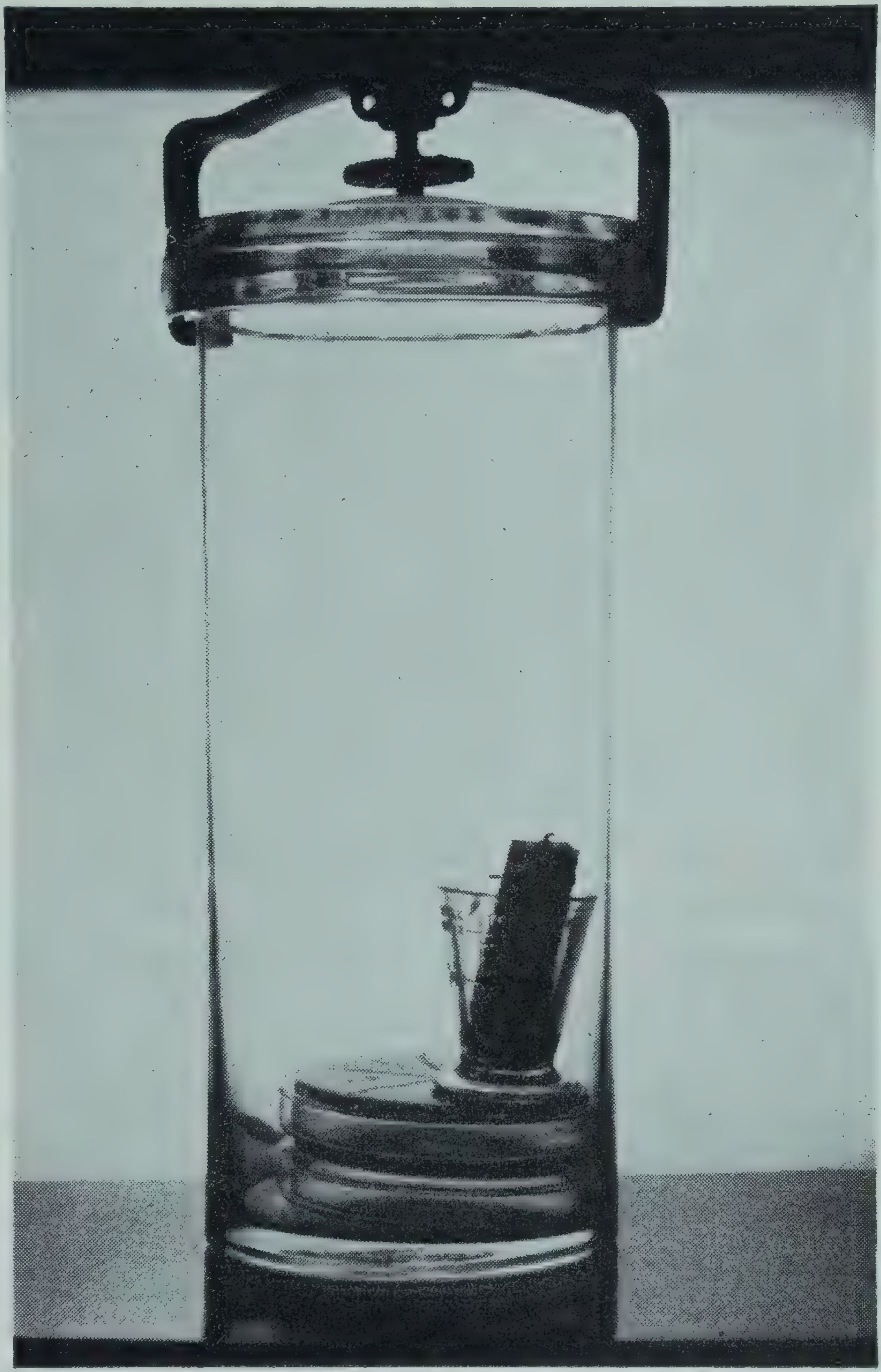

Fig. 3: Candle jar for bacteria that grow better in a mixture of carbon dioxide and oxygen, for example the meningococcus and gonococcus.

Secondary purulent meningitis may develop by extension from an adjacent infection, such as otitis media, mastoiditis, or sinusitis.

This may be either a localized or a diffuse meningitis, caused by streptococci, staphylococci, or pneumococci.

A localized meningitis may develop when a brain abscess extends out to the surface of the brain. In such a case, the causative organism is sometimes not immediately identified.

In Tuberculous Meningitis:

This form is secondary to some other focus in the body. A smear is made from the pellicle or from an artificial coagulum made by adding alcohol; however, the bacilli are not always found. Smears may have to be examined on more than one cerebrospinal-fluid specimen before finding the organism. In any event, appropriate culture and guinea-pig inoculation should be made to prove the diagnosis if all smears prove to be negative.

With the advent of streptomycin therapy, some cases have been kept alive for a few months or even longer. In these cases, it is particularly important to have a correct diagnosis so that a false cure will not be credited to the drug.

In Syphilitic Meningitis:

Syphilitic meningitis and other forms of syphilis are usually diagnosed by serological tests. Occasionally, the Treponema pallidum has been found in the cerebrospinal fluid by injecting it into a rabbit testicle. This is not a practical or universally accurate means of making a diagnosis, however.

SPUTUM

In the bacteriological examination of the sputum, it is important to have a morning specimen that is coughed up from the lungs and is not contaminated by organisms from the posterior nasopharynx and from the saliva. When such organisms as pneumococci, Hemophilus influenzae, or Klebsiella pneumoniae are present, they are seen in large numbers in the stained smear and suggest the etiological relationship. Normally, little or no sputum is coughed up.

Mycobacterium Tuberculosis:

The sputum is most frequently examined for the presence of the Mycobacterium tuberculosis. If a single specimen is negative, a twenty-four hour specimen should be concentrated and a stained smear of the sediment examined. If this is negative, the concentrate should be cultured and inoculated into a guinea pig.

Acid-fast bacilli other than Mycobacteria tuberculoses are sometimes found in the sputum. These are saprophytic, acid-fast organisms, such as the timothy Mycobacterium and the Mycobacterium butyricum. Consequently, in reporting a positive smear, it should be reported as "positive for acid-fast bacilli." If further confirmation is needed, the doctor should order a guinea-pig inoculation.

The Gaffky scale (Table 1) is of some value in grading the number of acid-fast bacilli in successive examinations. It is used for designating the number of bacilli in a smear.

TABLE 1: GAFFKY SCALE

Scale Number	*Acid-Fast Bacilli*
1.	Only 1 to 4 bacilli in a whole smear
2.	Only 1 bacillus on an average in many fields
3.	Only 1 bacillus on an average in one field
4.	Only 2 to 3 bacilli on an average in each field
5.	From 4 to 6 bacilli on an average in each field
6.	From 7 to 12 bacilli on an average in each field
7.	From 13 to 25 bacilli on an average in each field
8.	About 50 bacilli on an average in each field
9.	As many as 100 or more bacilli on an average in each field
10.	Enormous numbers in each field

Pneumococcus:

Pneumococci of various types are the cause of most cases of lobar pneumonia. Formerly, it was necessary to type these organisms in order that serum therapy of the same type might be instituted. With the use of the sulfonamides and penicillin in the treatment of pneumonia, typing is rarely requested. However, if an organism does not respond to chemotherapy or antibiotics, it is safer to have the organism typed so that serum therapy may be started if necessary.

Streptococcus Hemolyticus:

This streptococcus causes a few cases of lobar pneumonia and from 30 to 35 per cent of bronchopneumonia. It is readily identified in a smear stained by the Gram method. It must then be cultured on blood agar to determine whether or not it is hemolytic.

Spirochetes:

Pulmonary spirochetosis is apparently caused by Borrelia vincentii with or without Fusiformis dentium. These organisms can be found in a smear stained with methylene blue or gentian violet.

Miscellaneous Organisms:

Other organisms that cause lobar pneumonia are Klebsiella pneumoniae, Hemophilus influenzae, and the staphylococci.

In lung abscess, the sputum or material from a bronchus will show hemolytic streptococci, staphylococci, Escherichia coli, Hemophilus influenzae, Pseudomonas aeruginosa, or Klebsiella pneumoniae. In gangrene of the lung, spirochetes and fusiform bacilli are found. In bronchiectasis, there is a mixed infection including pyogenic bacteria, or spirochetes and fusiform bacilli.

Pasteurella pestis is found in the sputum in the pneumonic type of plague, and Bacillus anthracis is found in the sputum in cases of pulmonary anthrax.

FECES

Bacteriological examination of the feces is carried out chiefly to determine the causative organism of dysentery or to identify a carrier of pathogenic organisms. About one third of the weight of dried feces is made up of bacteria, most of which are dead. More than fifty different species of bacteria have been isolated from feces. Most of these are not pathogenic in the intestinal tract but can become pathogenic in the body cavities or tissues. In milk-fed infants, the majority of organisms are gram-positive bacilli of the Lactobacillus-acidophilus group, which give the stools a sour odor. In adults, the majority of organisms are gram-negative bacilli of the Escherichia-coli group. In addition to these, certain anaerobic organisms, such as streptococci, Clostridium perfringens, and Clostridium tetani may be present without causing any disturbance unless they enter the tissues.

In certain cases of food poisoning caused by the preformed toxins of staphylococci or Clostridium botulinum, the bacteriological examination of the feces does not help the clinician. Frequently, however, when the history of the relationship of ingestion of food to onset of diarrhea is not clear, it is safer to culture the stool.

PATHOGENIC ORGANISMS

Paracolon Bacillus:

The paracolon bacillus is characterized by late (from five to fourteen days) lactose fermentation. It should perhaps be considered as a transitional form between the lactose-fermenters and the nonlactose-fermenters and possibly be allied to the Shigella dysenteriae (Sonne). It is apparently pathogenic to a minor degree, since it has been found in association with mild enteric infection and has produced laboratory infections. I isolated a paracolon bacillus in one third of twenty-seven cases of infant diarrhea in a nursery outbreak. If this was the causative agent, it is highly pathogenic for newborn infants. Two adult, asymptomatic carriers were also found among the nurses who attended the infants.

Eberthella Typhosa:

The Eberthella typhosa is not usually found in the stool in typhoid fever until the Peyer's patches break down during the third week of the disease. Therefore, stool culture for this organism is of limited value during the early part of the disease.

Culture of the feces for the Eberthella typhosa should also be carried out after the patient has recovered from the disease to ascertain whether or not the person is going to be a chronic carrier. About 2 to 4 per cent of persons who have had typhoid fever become chronic carriers because of infection of the gallbladder or intrahepatic bile ducts. Females are more likely to become carriers than adult males, and children are least likely to become carriers.

Bacillus Paratyphosus:

Three different species of paratyphoid bacilli have been recognized as the cause of paratyphoid fever: Salmonella paratyphi (type

A), Salmonella shottmülleri (type B), and Salmonella hirschfeldii (type C). These organisms can be isolated from the blood and feces of persons who are infected. They are found also in the feces of carriers.

Salmonella-paratyphi (A) infection is usually mild and of shorter duration than typhoid fever. Salmonella shottmülleri (B) is readily differentiated from Salmonella paratyphi (A) but may be confused culturally and serologically with some Salmonella strains found in cases infected by food. Salmonella-shottmülleri (B) infection is more common in northern United States and northern Europe than is Salmonella paratyphi (A). Salmonella hirschfeldii (C) has been found in enteric infection in parts of Asia, Africa, southeastern Europe, and British Guiana.

Salmonella Group:

The Salmonella group is made up of a large number of antigenically related bacilli, in which are included the paratyphoid and typhoid bacilli. With the exception of Eberthella typhosa, Salmonella paratyphi (A), and Salmonella shottmülleri (B), the Salmonella organisms are considered primarily to be parasites of birds and animals. It seems likely that some of these organisms are transmitted from animals to man and from man to man. The members of this group can be isolated from the feces of infected persons and can be typed antigenically.

Dysentery Bacilli:

In the Bergey classification, the dysentery bacilli are members of a single genus, Shigella. The Shigella are the most common causes of true dysentery. The organism is not cultured from the blood, but it is cultured in the feces of persons with bacillary dysentery. In fact, this is the only way of differentiating the disease from dysentery caused by Endamoeba histolytica and Balantidium coli. Carriers of the chronic type apparently are much less common than those with the Eberthella typhosa.

Vibrio Comma:

Cholera is caused by the Vibrio comma, which is transmitted by the ingestion of contaminated water, food contaminated by water, or by direct contact with the dejecta of cholera patients. Bacteriological examination of the feces is necessary for the identification of the Vibriones, but they tend to disappear from the stool in four to fourteen days. Chronic and passive carriers may comprise as high as 7 per cent of the population in areas where cholera is endemic.

Mycobacterium Tuberculosis:

This organism may be identified in the feces of persons with tuberculous enteritis or in those who have swallowed the bacilli from pulmonary infection. A single negative culture or guinea-pig inoculation does not rule out tuberculous enteritis.

Bacillus Anthracis:

Anthrax is fortunately rare. It is due to the ingestion of Bacillus anthracis or its spores in raw or rare meats, or the swallowing of spores that are in the air. The organism is readily isolated from the stools of infected persons and can be differentiated easily from other spore-forming aerobic bacilli.

TRANSUDATES AND EXUDATES

Pleural, pericardial, and abdominal transudates are sterile, while exudates usually contain organisms.

Pleural Exudate:

The pleural exudate is usually caused by the Mycobacterium tuberculosis, which comes from a lesion on the surface of the lung. Since the organism is found in only 30 per cent of smear examinations, it is necessary to concentrate the fluid, culture it, and inoculate a guinea pig.

Other organisms which may be found in a pleural exudate are hemolytic and nonhemolytic streptococci, pneumococci, staphylococci, Escherichia coli, Klebsiella pneumoniae, or Borrelia vincentii with Fusiformis dentium.

Pericardial Exudate:

This may be secondary to pneumonia and would contain pneumococci, hemolytic streptococci, or Klebsiella pneumoniae. In rheumatic fever, a pericardial exudate may contain hemolytic streptococci. As a complication of septicemia, hemolytic streptococci or staphylococci are found. Rarely, such organisms as Pseudomonas aeruginosa, Escherichia coli, and Hemophilus influenzae are found.

Peritoneal Exudate:

Peritonitis is the cause of peritoneal exudates, and it may result from perforation of an appendix, intestine, gallbladder, peptic ulcer, typhoid ulcer, tuberculous ulcer, dysenteric ulcer, or diverticulum, and following perforating injuries to the abdomen. Pus from an infected uterine tube may leak out from the fimbriated end and infect the pelvic peritoneum with gonococci. Other organisms cultured are Escherichia coli, staphylococci, hemolytic and nonhemolytic streptococci, pneumococci, Clostridium perfringens, Pseudomonas aeruginosa, or Mycobacterium tuberculosis.

Aseptic peritonitis results from chemical irritation when sterile blood, urine, or bile escapes into the peritoneal cavity.

Joint Exudate:

The exudate in a joint may contain such pyogenic bacteria as streptococci, staphylococci, gonococci, pneumococci, Eberthella typhosa, or meningococci. The Mycobacterium tuberculosis sometimes causes a joint exudate (tuberculous arthritis). The organism is difficult to find by means of a direct smear; hence, it is preferable to concentrate, culture, and then inoculate a guinea pig. Chronic suppurative arthritis may be caused by staphylococci, streptococci, Escherichia coli, or Pseudomonas aeruginosa. There may be a mixed culture with Proteus vulgaris and Corynebacterium diphtheriae present as secondary invaders.

ORIFICES AND TISSUES

EYES

In examining material from the eyes for bacteria, it is preferable to prepare both smears and cultures. The normal conjunctivas may contain Corynebacterium xerose, Staphylococcus albus, and type-IV pneumococcus.

In cases of nonspecific conjunctivitis, the most common organisms are staphylococci, pneumococci, streptococci, and Hemophilus influenzae. In infectious conjunctivitis (pink-eye), the usual cause is the Hemophilus of Koch-Weeks. In blepharoconjunctivitis, the Hemophilus of Morax-Axenfeld is the usual offender. Pseudomembranous conjunctivitis is caused by a streptococcus or the Corynebacterium diphtheriae. The gonococcus causes a very purulent type of conjunctivitis. It is important to recognize that the pneumococcus becomes gram-negative when it is dead and may be confused with the gonococcus.

MOUTH AND THROAT

The mouth and throat contain a variety of organisms, which might be considered the normal flora. The presence of these bacteria makes the study of mouth and throat infections more difficult.

In Vincent's infection of either the mouth or throat, Borrelia vincentii and Fusiformis dentium are found on the direct smear. A few of these organisms may be found in the normal mouth; but in Vincent's infection, they are present in large numbers. Vincent's organisms, together with streptococci and staphylococci, are found in gangrenous stomatitis (noma).

In syphilitic lesions of the mouth and throat, a darkfield examination may reveal Treponema pallidum. This should be confirmed by serological examination because of the possibility of confusing Treponema pallidum with Treponema microdentium.

Gingivitis may be caused by bacterial infection, Borrelia vincentii, and Fusiformis dentium, administration of mercury or bismuth, agranulocytosis with secondary infection, food impactions, tartar deposits, and ill-fitting crowns. Secondary infection usually takes place when the gums are injured. These secondary invaders are streptococci, staphylococci, pneumococci, or Borrelia vincentii with Fusiformis dentium.

Infection of the throat and tonsils is usually caused by streptococci, Corynebacterium diphtheriae, staphylococci, pneumococci, or Borrelia vincentii with Fusiformis dentium. Reports on examination of the direct smear concerning the presence or absence of Corynebacterium diphtheriae should be made immediately so that appropriate serum therapy may be instituted if it is positive. If the smear is negative, an early report should be made from the culture.

Peritonsillar abscess and retropharyngeal abscess are caused by staphylococci or hemolytic streptococci.

Bacteriological examination of the posterior nasopharynx is important in the detection of meningococcic carriers. The diagnosis of pertussis may be confirmed by culturing Hemophilus pertussis from a cough plate or from a throat culture.

NOSE AND SINUSES

In nasal diphtheria, the organism can be demonstrated by smear and culture. Infection of the hairs in the anterior part of the nose and abscess of the septum are caused by Staphylococcus aureus.

The Mycobacterium leprae is found in smears from the nose of a large proportion of persons with leprosy, even though nasal lesions are not demonstrable. The Mycobacterium tuberculosis is rarely found in the nose unless a tuberculous process has ruptured into the nose.

In primary syphilitic lesions of the nose, a darkfield examination is indicated for the demonstration of Treponema pallidum.

Infection of the accessory sinuses is usually due to a mixed infection, although pure cultures are occasionally found. The organisms commonly found are Staphylococcus aureus, Streptococcus hemolyticus, Diplococcus pneumoniae, Klebsiella pneumoniae, Hemophilus influenzae, Neisseria catarrhalis, and Pseudomonas aeruginosa.

EARS

Infection of the external auditory canal is usually a furunculosis caused by Staphylococcus aureus or albus. Fungous infections of the auditory canal are caused by molds of the Penicillium or Aspergillus genus.

Acute otitis media is caused by Staphylococcus aureus, Streptococcus hemolyticus, Diplococcus pneumoniae, Corynebacterium diphtheriae, Klebsiella pneumoniae, or Hemophilus influenzae. If the infection becomes chronic, there is a mixture of one or more of the above-mentioned organisms with saprophytes. If the mastoid becomes infected, it is usually owing to the same organism that causes the otitis media.

GENITAL ORGANS AND URETHRA

Careful bacteriological examination of lesions and discharges of the genital organs and urethra is very important in the diagnosis of venereal disease and other infections.

Syphilis:

Any ulceration or abrasion of the genitalia of either sex should suggest the possibility of syphilis, and it warrants a darkfield examination for Treponema pallidum. With early diagnosis and immediate treatment, the hope of cure is much greater. The examination should be made, however, by an experienced person who recognizes the possibility of confusing Treponema pallidum with other spirochetes, particularly, Treponema refringens. The darkfield may be negative in lesions that have been treated with chemicals and in lesions that have started to heal. Aspiration of a groin gland may yield a positive darkfield under these conditions.

Chancroid:

Smear and culture will demonstrate Hemophilus ducreyi in the majority of cases. A darkfield should also be done to rule out a primary syphilitic lesion.

Gonorrhea:

Acute gonorrhea occurs as a urethritis in both sexes. In adult females, Bartholin's glands, Skene's glands, the vagina, cervix, and uterine tubes may also be involved. In the male, the prostate and seminal vesicles are sometimes invaded by the gonococcus.

In young girls and female infants, gonorrheal vaginitis is particularly contagious; therefore, such patients should be carefully isolated from other children.

A smear of the purulent discharge stained by the Gram method shows many pus cells, some of which contain gram-negative, biscuit-shaped diplococci. This is satisfactory for a diagnosis in most instances. In suspected cases, where the smears give negative results, less than 10 per cent carbon-dioxide tension will frequently grow the gonococcus.

In examining smears from the male urethra, staphylococci and gram-negative diplococci other than gonococci are frequently present as nonpathogenic bacteria. In the vagina, Neisseria catarrhalis, staphylococci, diplococci, streptococci, pneumococci, Corynebacterium diphtheriae, Bacillus döderleini, and other lactobacilli constitute the normal flora. Neisseria catarrhalis and other gram-negative diplococci must be differentiated from gonococci.

Lymphopathia Venereum:

This is a venereal disease that is caused by a filtrable virus. It occurs mostly among negroes, especially in the southern part of the United States. The Frei test is the best method of diagnosing this disease.

Granuloma Inguinale:

This disease is thought to be caused by the Klebsiella granulomatis which looks something like the protozoon of the genus Leishmania. Smears from the ulcerating buboes, stained with Wright's stain, will reveal the intracellular Donovan bodies.

Vaginitis:

Nongonorrheal vaginitis may be caused by Trichomonas vaginalis, fungous infections, and Oxyuris vermicularis that migrate from the anus.

Balanitis:

This infection is frequently the result of phimosis, in which staphylococci and streptococci are found. Occasionally, a gangrenous type of balanitis is caused by Borrelia vincentii with Fusiformis dentium.

Prostatitis:

In addition to infection with gonococci, the prostate may be infected with staphylococci, streptococci, Escherichia coli, and the Mycobacterium tuberculosis.

Epididymitis:

Infection of the epididymis is caused by gonococci, Mycobacterium tuberculosis, or mixed pyogenic organisms.

Orchitis:

Acute infection of the testicle may be due to the virus of mumps, staphylococci, streptococci, or Escherichia coli. A mixed infection may cause a chronic form of orchitis or an abscess. Gumma of the testicle is sometimes seen, and it is not an uncommon complication of syphilis.

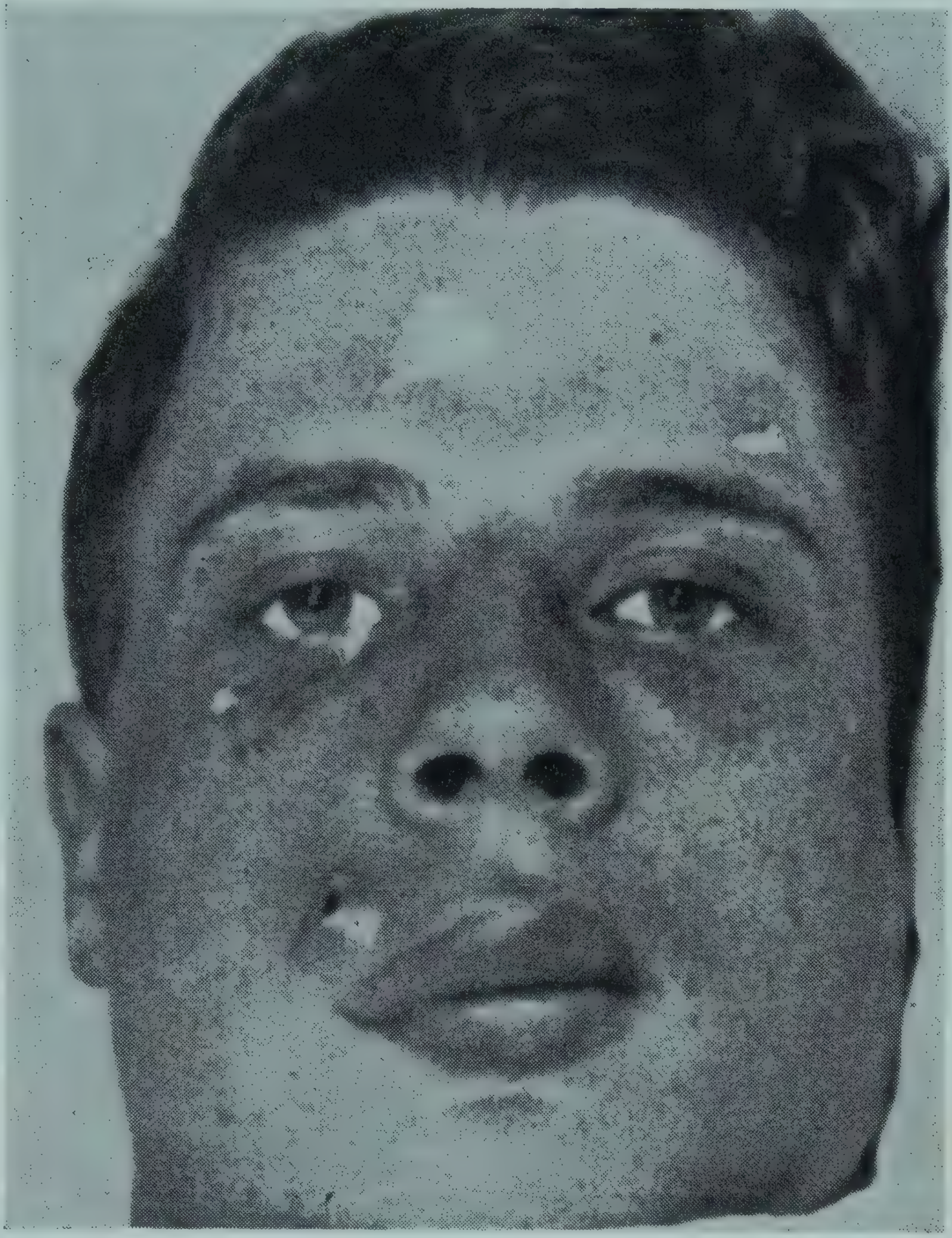

Fig. 4: Carbuncle above upper lip (Staphylococcus aureus).

SKIN

The intact skin protects the body from infection, but it contains many bacteria that invade immediately when there is a break in the continuity.

Postoperative Wound Infection:

Wound infection is caused by air-borne bacteria or bacteria of the skin that have not been killed. It is caused most commonly by staphylococci or streptococci. The normal body defenses take care of the few bacteria that are present in every surgical wound; therefore, very few clean wounds develop postoperative infections.

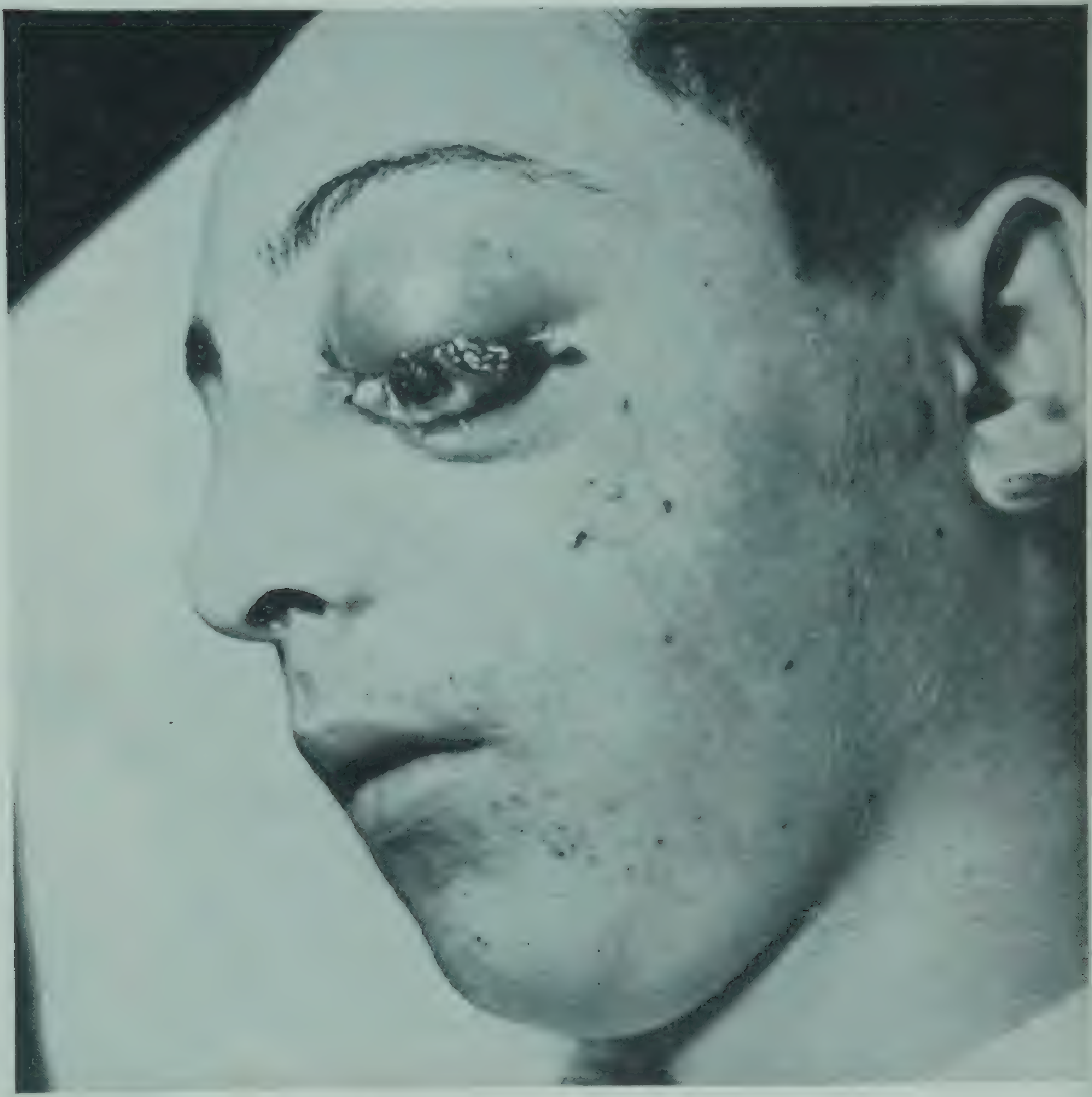

Fig. 5: Staphylococcus-aureus infection of eyelid.

Traumatic Wound Infection:

Traumatic wounds become infected with staphylococci, streptococci, or a mixture of organisms that may have been forced into the wound. Such organisms include the Clostridium perfringens and Clostridium tetani, both of which are extremely serious. Routine anaerobic cultures will detect the Clostridium perfringens within thirty hours if it is present. It is impossible, however, to find the Clostridium tetani in a smear. It is necessary to carry out anaerobic cultures and guinea-pig inoculation, and even then the organism is rarely found.

Staphylococcus Infection:

Carbuncles are caused by Staphylococcus aureus, while furuncles are caused either by Staphylococcus albus or aureus. Paronychias and felons are also caused by Staphylococcus aureus.

Streptococcus Infection:

Erysipelas is caused by a group-A hemolytic streptococcus. Occasionally, a carbuncle or a cellulitis is caused by a hemolytic streptococcus.

Anthrax Infection:

Examination for Bacillus anthracis should be carried out in the investigation of pustules among those who are employed in handling hides. Both smears and cultures of the lesions are diagnostic.

Pasturella-Tularensis Infection:

Ulcers occurring on the hands of those who handle rabbits or rabbit meat should be thoroughly investigated by cultural methods and guinea-pig inoculation for the detection of Pasturella tularensis. Of course, other organisms, including Treponema pallidum, should be considered.

BONES

Osteomyelitis:

This infection is caused most commonly by Staphylococcus aureus. Less frequent infecting organisms are the Streptococcus hemolyticus, Diplococcus pneumoniae, Eberthella typhosa, Escherichia coli, or Mycobacterium tuberculosis. If no organisms are found by direct smear, the Mycobacterium tuberculosis should be considered, and appropriate cultures or guinea-pig inoculation should be carried out.

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CHAPTER 14

Rickettsias and Viruses

RICKETTSIAS

In 1908, Ricketts described bacillary bodies in the blood of patients ill with Rocky Mountain spotted fever. In his honor, the name "Rickettsia" was given to certain organisms that may represent the link between bacteria and viruses. In microscopic visibility and inability to pass through certain filters, they resemble small bacteria, but in other characteristics they resemble viruses.

Rickettsias have coccal, diplococcal, and bacilluslike forms and are nonmotile. They vary from 0.3 to 2 microns in length and have a diameter of about 0.3 of a micron. They apparently do not have a capsule; they are gram-negative and stain purple with Giemsa's stain.

These organisms require living material on which to grow. They will grow on the chorioallantoic membrane of the chick embryo, in guinea pigs, and in the rectal canals of lice. For the most part, they grow intracellularly.

Weil-Felix Reaction:

In 1916, Weil and Felix isolated a strain of Proteus bacillus that was agglutinated by the serum of typhus patients. This reaction

TABLE 1: THE RICKETTSIAL DISEASES*

	Typhus Fever				*Tsutsugamushi Disease*	*São Paulo Fever*	*Rocky Mountain Spotted Fever*		*Trench Fever*	*Rickettsial-Pox*
	Epidemic Typhus and Brill's Disease	*Endemic Typhus*	*Shop Typhus*	*Scrub Typhus*			*Western*	*Eastern*		
Etiological Agent . . .	Rickettsia prowazeki	Rickettsia prowazeki (variant?)	Rickettsia prowazeki (variant?)	Rickettsia prowazeki (variant?)	Rickettsia nipponica	Rickettsia rickettsi(?)	Rickettsia rickettsi	Rickettsia rickettsi	Rickettsia quintana	Rickettsia akari]
Natural reservoir. .	Man (?)	Rat	Rat	Rat	Mice and rats	Rodents	Ticks and rodents	Ticks, rodents, and dogs (?)	Man (?)	(?)
Vector	Lice	Fleas	Fleas	Mites	Mites	Ticks	Ticks	Ticks	Lice	(?)
Scrotal reaction in guinea pigs	Slight	Marked	Marked	None	None	Marked	Marked	Marked	None	(?)
Weil-Felix reaction with Proteus . .	OX_{19}	OX_{19}	OX_{19}	OXK	OXK	OX_{19}, OX_2, OXK	OX_{19}, OX_2, OXK	OX_{19}, OX_2, OXK	(?)	Complement-fixation test positive

*Modified from Belding, D. L., and Marston, A. T.: Medical Bacteriology. D. Appleton-Century Co., New York, 1938, p. 434.

concerns only the O type of agglutination rather than both O and H agglutination as in the Widal reaction. The strain of Proteus used for diagnosing typhus fever is OX_{19}; and two other strains, OX_2 and OXK, are used to differentiate other rickettsial diseases.

The rickettsial diseases of man are typhus fever, Rocky Mountain spotted fever, tsutsugamushi disease, trench fever, and rickettsialpox. A resumé of the rickettsial diseases is found in Table 1.

Typhus Fever:

Epidemic typhus fever is an infectious febrile disease with a macular rash, headache, and muscle pains. It is caused by Rickettsia prowazeki, which is transmitted from man to man by the body louse (Pediculus corporis).

The diagnosis is made by a positive Weil-Felix reaction with Proteus OX_{19} in serum dilutions of 1:80 or higher. Although not used routinely, the intra-abdominal inoculation of guinea pigs with from 1 to 5 cc. of the patient's blood or the ground-lice bodies will produce a febrile disease and characteristic lesions in the brain (thromboses, hemorrhages, and perivascular lymphocytes and leukocytes). Very little reaction occurs in the scrotum.

Endemic typhus is a milder form of the disease. It is also caused by Rickettsia prowazeki, but it is transmitted to man by the rat flea, (Xenopsylla cheopis), which becomes infected from rats. It may be transmitted from man to man by the body louse after it has become established in a human. The diagnosis is made by the Weil-Felix reaction using Proteus OX_{19}. The reaction in guinea pigs is similar to that of epidemic typhus except that the scrotal reaction is marked, and Rickettsias can be isolated from the tunica vaginalis where they are found in the cytoplasm of cells.

Rocky Mountain Spotted Fever:

This is an acute, febrile disease with a sudden onset, chill, and rapid rise in temperature with morning remissions. A macular rash appears on the third or fourth day in the mouth and pharynx, as well as on the skin. There are two forms of this disease: the western, transmitted by Dermacentor andersoni; and the eastern, transmitted by Dermacentor variabilis. Both types of the disease are caused by Rickettsia rickettsi.

The diagnosis is made by the Weil-Felix reaction, which gives a positive reaction with Proteus OX_{19}, OX_2, and OXK. Blood injected into the guinea pig causes marked scrotal reaction with necrosis and thrombosis. The organisms in tissue cultures are found in the nuclei, as well as in the cytoplasm.

Tick-bite fever of South Africa, boutonneuse fever of the Mediterranean area, and São Paulo fever of Brazil are apparently closely related to Rocky Mountain spotted fever.

Trench Fever:

Trench fever, publicized in the first World War, is characterized by sudden onset, fever, headache, and pain in muscles and eyeballs. It is caused by Rickettsia quintana, which is transmitted from man to man by the body louse.

Diagnosis is made on clinical signs and symptoms because the Weil-Felix reaction and guinea-pig inoculation are not usually positive.

Tsutsugamushi Disease:

This is an acute, febrile, exanthematous disease, which occurs in the river valleys of Japan. The disease is caused by Rickettsia nipponica, which is transmitted to man through the larval mite (Trombicula akamushi). The natural reservoir is the field mouse, from which the larval mite becomes infected. Establishment of the diagnosis is aided by a positive Weil-Felix reaction with Proteus OXK and a positive reaction in rabbits after intraocular injection.

This disease appears to be related to scrub typhus, which is found in the Malay states and is transmitted from infected rats to man by mites.

Rickettsialpox:

According to Greenberg and associates,[1] rickettsialpox is a new nonfatal disease of rickettsial origin. It is characterized by an abrupt onset of chills, fever, sweats, and backache, followed immediately or in a few days by a maculopapular and maculovesicular rash.

About a week prior to the onset of fever, there is an initial skin lesion that has the appearance of a blind boil. The disease thus far has been reported only from certain boroughs in New York City.

A strain of Rickettsia was isolated from the blood of two patients. This was identical with a strain isolated from two pools of rodent mites, Allodermanyssus sanguineus. "Rickettsialpox" has been proposed for the name of the disease,[2] and "Rickettsia akari" has been suggested for the name of the causative organism.[3]

Laboratory Findings:

1. An antigen prepared from one strain of Rickettsia akari gave a positive complement-fixation test with serum of patients who had recovered from the disease. Significant rises in titer were noted in a few cases tested early, and again late in the disease.
2. There was a moderate leukopenia in twenty-one cases reported. The white count ranged from 2400 to 7500 per cubic millimeter. The count usually returned to normal about two weeks after the onset of fever.
3. The differential count was usually normal, but occasionally showed a relative lymphocytosis.
4. Erythrocyte counts and hemoglobin determinations were normal.

VIRUSES

Viruses are difficult to define, but they are apparently living agents, which are invisible under the microscope and are capable of passing filters that retain ordinary bacteria. This statement must be qualified because some of the larger viruses may be observed under the microscope in stained preparations. An important characteristic of viruses is the fact that living cells are necessary for their cultivation. They live in the host within cells or in intimate relation with cells, but this does not set them apart from the rickettsias, certain bacteria, and protozoa. Viruses have been estimated to range from 10 to 250 millimicrons in size. The virus of poliomyelitis is among the smallest, while that of psittacosis is among the largest.

With our present limited knowledge of viruses, no satisfactory classification can be made because any permanent classification must be based upon the causative agent. One classification suggested is based upon the affinity of viruses for certain organs: dermatotropic (affinity for the skin), neurotropic (affinity for the nervous system), viscerotropic (affinity for abdominal or thoracic viscera), pneumotropic (affinity for lungs), affinity for respiratory mucous membranes, and miscellaneous.

Following is a classification of viral diseases of man based upon these peculiarities:

1. Dermatotropic viral diseases
   - *a.* Variola (smallpox)
   - *b.* Vaccinia (cowpox)
   - *c.* Varicella (chicken pox)
   - *d.* Herpes
     - (1) Herpes febrilis
     - (2) Herpes genitalis
   - *e.* Herpes zoster
   - *f.* Lymphogranuloma venereum
   - *g.* Molluscum contagiosum
   - *h.* Verrucae (warts)
   - *i.* Rubeola (measles)
   - *j.* Rubella (German measles)
2. Neurotropic viral diseases
   - *a.* Anterior poliomyelitis
   - *b.* Epidemic encephalitis
   - *c.* Other forms of encephalitis
     - (1) Japanese encephalitis
     - (2) St. Louis encephalitis
     - (3) Australian X encephalitis
     - (4) Secondary encephalitis
     - (5) Russian encephalitis
     - (6) Louping ill
   - *d.* Lymphocytic choriomeningitis
   - *e.* Rabies
3. Viscerotopic viral diseases
   - *a.* Yellow fever
   - *b.* Hepatitis
   - *c.* Rift Valley fever
4. Pneumotropic viral diseases
   - *a.* Epidemic influenza
   - *b.* Primary atypical pneumonia
   - c. Psittacosis
5. Viral diseases of respiratory mucous membranes
   - *a.* Common cold
   - *b.* Epidemic parotitis (mumps)

6. Miscellaneous
    a. Trachoma
    b. Dengue fever
    c. Phlebotomus fever (sandfly fever)
    d. Epidemic keratoconjunctivitis
    e. Foot-and-mouth disease

DIAGNOSIS

Ordinary bacteriological methods for the diagnosis of viral diseases are not satisfactory because of their fastidious cultural requirements and their invisibility under the standard microscope. At present, some viral diseases must be diagnosed by the clinical manifestations, since our present knowledge is not sufficient to make a laboratory diagnosis in these cases. Other viral diseases can be diagnosed only in those laboratories especially equipped for viral investigations. In some diseases, these procedures are too long to be of practical use to the clinician. Diseases will be discussed below that lend themselves to any type of laboratory diagnosis.

DERMATOTROPIC VIRAL DISEASES

Variola:

In variola, or smallpox, the intracellular bodies are known as "Guarnieri's bodies." Paschen later described corpuscular elements in vaccine lymph measuring from 100 to 200 micromicrons. These are regarded as particles of virus. This virus can be grown on the chorioallantoic membrane of the chick embryo.

A test devised by Paul involves inoculating the scratched cornea of a rabbit with material from the skin lesion. In the presence of the virus, an umbilicated papule will develop. Intradermal inoculation of material from a skin lesion will develop a papule in four days. An immunized rabbit should be used as a control.

Vaccinia:

The presence of the vaccinia (cowpox) virus may be demonstrated by the neutralization test, complement-fixation test, and hemagglutinin-inhibition test.

Neutralization Test: The neutralization test is based upon the fact that certain viral infections stimulate the production of antibodies in the host. These antibodies are able to neutralize the specific virus, as shown by the inability of the serum-virus mixture to cause recognizable disease when inoculated into susceptible animals. Serial dilutions of serums from the acute and convalescent phases are set up in parallel, and the same amount of virus is added to each tube. After incubation of the virus-serum mixtures, animals are inoculated with samples from each and are followed for the development of disease. In a positive test with serum taken during convalescence, there is a definite neutralizing, or protective, effect as compared with that of the acute phase.

Complement-Fixation Test: Some viruses have the property of stimulating complement-fixing antibodies in the host as a sequel of infection. To establish a diagnosis, Cheever[4] states that it is essential to prove that antibodies appear or show a significant rise in titer during the course of the disease. It is, therefore, essential that two specimens of blood be examined, one soon after onset and the other several days later. The antigen employed represents the virus or its products. Since the source of antigen is infected tissue or fluid, it is necessary to include in each test an additional control, consisting of normal tissue or fluid, to rule out nonspecific reactions.

Hemagglutinin-Inhibition Test: The viruses of vaccinia and certain other viral diseases have the property of agglutinating certain avian and mammalian red-blood cells. Patients with viral diseases, including vaccinia, develop antibodies that inhibit the ability of the virus to agglutinate the erythrocytes.

After the hemagglutinating activity of the viral agent has been determined, four minimal agglutinating doses of virus are added to increasing dilutions of the serum to be tested, and fowl or mammalian red-blood cells are added. In this manner, the ability to inhibit hemagglutination of two or more specimens of serum from a suspected case may be compared. When there is a definite rise in titer, a definite diagnosis can be made.

Since the cowpox virus is used to vaccinate against smallpox, and since vaccinia does not occur naturally, the diagnosis of the disease does not present a problem. The above tests are outlined, however, because they are applicable to certain other viral diseases.

Varicella:

Elementary bodies, which are agglutinated in convalescent serum, are regarded as the causative agent. Intranuclear, acidophilic, inclusion bodies may also be present. Rabbits inoculated in the testicle with material from a lesion will develop a lesion containing the elementary bodies.

Herpes:

In herpes febrilis, elementary coccoid bodies occur in the vesicular fluid and as intranuclear acidophilic bodies in the cells. The presence of the virus may be demonstrated by inoculating the scarified rabbit cornea with the fluid from a lesion. If the herpes virus is present, the rabbit develops a severe keratitis very rapidly.

Herpes genitalis is similar to herpes febrilis, except for the location of the lesions and a milder form of keratitis in the inoculated rabbit.

Herpes Zoster:

This is an inflammatory process of the sensory nerve roots with pain and a pustular eruption. Elementary bodies and intranuclear inclusions can be seen in the affected cells. Animal inoculation is not satisfactory.

Lymphogranuloma Venereum:

The intracutaneous test of Frei is preferred in the diagnosis of this disease. Material from a mouse brain infected by intracerebral inoculation is introduced into the skin and read in forty-eight hours. A positive test shows redness, swelling, and a central area of necrosis.

Molluscum Contagiosum:

This is a chronic viral infection of the skin, in which there are tiny pink or pearl-gray nodules. The molluscum bodies are about 250 millimicrons in diameter and may be seen early as tiny vacuoles in the cytoplasm. Later, visible viral bodies develop in the vacuoles, particularly after they coalesce. The disease cannot be reproduced in animals, but typical lesions develop in man following the intradermal injection of ground and filtered material.

Verrucae:

Verrucae, or warts, are caused by a filtrable virus, the bodies of which are located within the nuclei of the epithelial cells.

Rubeola:

Nasal washings will infect man and monkeys. The viral bodies of measles have been demonstrated in the corneal epithelium of rabbits inoculated with appropriate material.

Rubella:

German measles has not been proved to be a viral disease in the laboratory because the virus has not been demonstrated directly or indirectly. The epidemiology is similar to that of other viral diseases, and so it is at present considered in this category.

NEUROTROPIC VIRAL DISEASES

Anterior Poliomyelitis:

This disease does not lend itself to laboratory diagnosis of the presence of the virus. Neutralization tests using the monkey have been carried out by Aycock and Kramer,[5] and by Kolmer and Rule,[6] but the multiplicity of viral strains that react weakly, if at all, render this test impractical for routine diagnosis. The diagnosis depends chiefly upon clinical examination and the cytology of the cerebrospinal fluid (see Chapter 26, p. 502).

Encephalitis:

It is assumed that encephalitis is a viral infection because of its marked similarity to other proved viral infections. Considerable evidence has been accumulated to prove that the viruses of epidemic encephalitis and of herpes febrilis are the same agent. Neutralization tests and complement-fixation tests are useful in the diagnosis of the various types of encephalitis, but there is considerable delay in proving the diagnosis.

Encephalitis lethargica is a sporadic type of disease. The epidemic types of encephalitis are the St. Louis form, Japanese form, equine encephalitis, and Australian-X disease.

Postinfectious encephalitis is seen sometimes following influenza, measles, German measles, mumps, vaccinia, and variola. For the cerebrospinal-fluid changes in encephalitis see Chapter 26 (p. 502).

Lymphocytic Choriomeningitis:

This is an acute aseptic meningitis, characterized by an acute onset, stiff neck, headache, and vomiting. The patient makes a complete recovery in about two weeks with only symptomatic treatment. The virus is estimated to be between 70 and 80 micromicrons in diameter. Eosinophilic intranuclear agents have been reported in the cells of the meninges and brain cortex. It is pathogenic for monkeys, guinea pigs, and mice. Positive neutralization tests and positive complement-fixation tests are found in this disease. Cytological and chemical studies of the cerebrospinal fluid are used to make the diagnosis and to differentiate it from other types of meningitis (see Chapter 26, p. 503).

Rabies:

The rabies virus is present in the saliva of rabid animals; consequently, a bite by such an animal or inoculation of a scratch with saliva constitutes a source of infection. The diagnosis of rabies in man, aside from clinical signs, is made indirectly by examining the brain of the suspected animal: Sections are taken from the cerebral cortex near the crucial sulcus, from the cerebellar cortex, and from the hippocampus major. These are crushed on a slide, stained, and examined for Negri bodies. If Negri bodies are present, a positive diagnosis can be made. If negative, the tissues are fixed and processed in the usual way for paraffin sections. They are again examined for Negri bodies.

Rabbits or mice may be inoculated intracerebrally with a suspension of ground-up brain material. In the presence of the rabies virus, the animals develop progressive paralysis, and appropriate preparations from their brains will contain Negri bodies.

VISCEROTROPIC VIRAL DISEASES

Yellow Fever:

This is an acute febrile disease of tropical countries, transmitted by the female Aedes aegypti and other species of mosquito. During the first three days of the disease, the infection can be transmitted to monkeys and mice by inoculation with the patient's blood. Later, the laboratory diagnosis depends upon the demonstration of immune bodies in the serum by mouse-protective tests and by precipitation tests.

Hepatitis:

In 1941 and 1942, several million doses of yellow-fever vaccines were given to members of the United States armed forces. In 1942, many cases of jaundice were observed, more in the Army than in the Navy. They followed inoculations by a period of a few weeks to three or more months. The jaundice was due to hepatitis, which was later found to be due to human serum used in the preparation of the material. Although a final statement cannot be made as to the causative agent, it was presumably an icterogenic virus from a carrier.

This type of hepatitis is similar to the epidemic and sporadic cases of hepatitis seen in the armed forces and in civilian life. Both are presumably due to a virus, but as yet they cannot be readily diagnosed by laboratory procedures.

Rift Valley Fever:

This is a disease of sheep in Africa, characterized by vomiting and diarrhea. In man, it resembles dengue with fever and prostration. The virus is from 23 to 35 micromicrons in diameter. Acidophilic, intranuclear, inclusion bodies can be demonstrated in liver cells, and the virus can be grown in tissue cultures. The virus is infectious for mice, monkeys, and ferrets.

Pneumotropic Viral Diseases

Epidemic Influenza:

Smith and associates,[7] produced the disease in ferrets by intranasal inoculation with nasopharyngeal washings. This marked the beginning of intensive investigation of the disease. Strains of the influenza virus fall into two antigenically different groups, which are called "A" and "B." Some cases of epidemic influenza yield neither of these viruses and fail to develop an antibody against either, thus indicating that one or more types exist that have not yet been isolated.[8]

Neutralization tests, complement-fixation tests, hemagglutinin-inhibition tests, and skin tests are useful in diagnosing the disease. The last three tests can differentiate between types A and B and other influenza viruses.

Primary Atypical Pneumonia:

At least six viral agents other than the influenza virus, psittacosis virus, and lymphocytic-choriomeningitis virus have been isolated by various investigators in the search for the cause of primary atypical pneumonia. Further observations are necessary before the causative agent can be determined.

Peterson, Ham, and Finland[9] observed that serum from patients with primary atypical pneumonia will agglutinate human erythrocytes in the cold at 39.2° F. (4° C.) but not at 98.6° F. (37° C.). The agglutination is reversible. The titer begins to rise during the second week of illness and persists for two months or more. This rise in titer is helpful in the diagnosis of the disease. During the illness, the titer may rise from 1:14 to 1:224 or higher.

It must be remembered that mumps, measles, scarlet fever, and rheumatic fever may cause an increase in cold-agglutinin titer, but the clinical syndromes differentiate these diseases from primary atypical pneumonia.

Psittacosis:

This is an infectious disease of birds, particularly parrots, characterized by nasal discharge, diarrhea, weakness, and moping. Man is infected by inhaling the virus from the discharges from parrots or parakeets. In man, an atypical type of pneumonia with fever and prostration is the characteristic finding.

If the blood or sputum from an infected person is injected into the peritoneal cavity of a mouse, it will produce a characteristic infection that will kill the mouse in a week. Necrosis of the liver and spleen is found, and intracellular bodies may be demonstrated. The complement-fixation test is positive.

VIRAL DISEASES OF RESPIRATORY MUCOUS MEMBRANES

Common Cold:

The transfer of acute coryza to chimpanzees and human beings by means of filtered nasal washings makes it practically certain that the causative agent is a virus. The virus has been grown upon the chorioallantoic membrane of the chick embryo and in tissue culture. The symptoms are so obvious that laboratory diagnostic procedures are unnecessary. The important problem is a method of preventing the disease.

Epidemic Parotitis:

The virus of epidemic parotitis, or mumps, is resistant to desiccation, freezing, and glycerol and is pathogenic to monkeys and cats. The complement-fixation and skin tests have been demonstrated to be positive in this disease, but practically the diagnosis is made by the clinician.

MISCELLANEOUS

Trachoma:

This is a chronic disease of the eye with papillary hypertrophy of the conjunctiva, pannus formation in the cornea, and, secondarily, cicatrization of the conjunctiva and destruction of areas in the lids and cornea. Intracellular bodies are found regularly in material so that their presence is a simple means of making a diagnosis or at least differentiating it from other conditions in the eye. Rake and Jones[10] have pointed out the similarity of these bodies to those of psittacosis and lymphogranuloma venereum.

Dengue Fever:

This disease is also known as "breakbone fever," and it is characterized by fever, headache, pain in eyes and muscles, and sometimes a maculopapular rash. It is transmitted by Aedes-aegypti and Aedes-albopictus mosquitoes. The virus is present in the patient's blood on the day before the onset and during the first three days of the disease. The virus has been cultivated on the chorioallantoic membrane of chick embryos by Shortt and co-workers.[11]

Phlebotomus Fever:

This disease is also known as "pappataci fever," "sandfly fever," and "three-day fever." It is a febrile disease of three or four days' duration with general malaise. It is supposedly transmitted by the sandfly, Phlebotomus papatasii. The virus is present in the blood of man a day or two before the onset of fever and one day afterward. No susceptible animal has been found; thus, experimental work has been carried out on human volunteers. Human beings are experimentally infected with intravenous inoculation of blood from patients.

Epidemic Keratoconjunctivitis:

This eye infection was presumably imported from Hawaii. It is highly infectious, has very little exudate, but does cause headache and swelling of regional lymph nodes. A filtrable agent was isolated and cultured in tissues by Sanders.[12] A neutralizing antibody was demonstrated for this agent, and the disease was produced in a human volunteer.

Foot-and-Mouth Disease:

This is a disease of cattle characterized by a vesicular lesion of the skin or mucous membrane. The proved cases in humans had transient vesicles on the hands and feet. Only a few cases in man have been proved by animal inoculation.

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CHAPTER 15

Mycological Examinations

The laboratory diagnosis of fungous infections of the skin can usually be made by direct microscopic examination of skin scrapings, hairs, pus, or sputum. This material must be prepared by adding a few drops of from 10 to 40 per cent sodium or potassium hydroxide to free the fungus. Cultures are necessary to identify some fungi, while animal inoculation is helpful in others.

The botanical classification of fungi is, to date, unsatisfactory because there is much disagreement among students of the subject in regard to all schemes that have been proposed. No botanical criteria have, as yet, been created by which the genera and species of this order of vegetable life can be classified satisfactorily according to cultural, biological, or microscopic characteristics.

Although classifications should be made on the basis of etiology, it seems more practical to group the mycoses according to clinical variations as suggested by Jacobson:[1]

1. Primary cutaneous mycosis (dermatomycosis) with no definite systemic involvement
2. Primary cutaneous and/or mucous-membrane infections with frequent systemic involvement
3. Primary systemic infections with occasional instances of skin or mucous-membrane involvements

DERMATOMYCOSES

The organisms that cause these dermatoses belong to fungi imperfecti, since no process resembling sexual reproduction has ever been observed in them. They belong to the following genera, and the diseases which they cause also are listed:

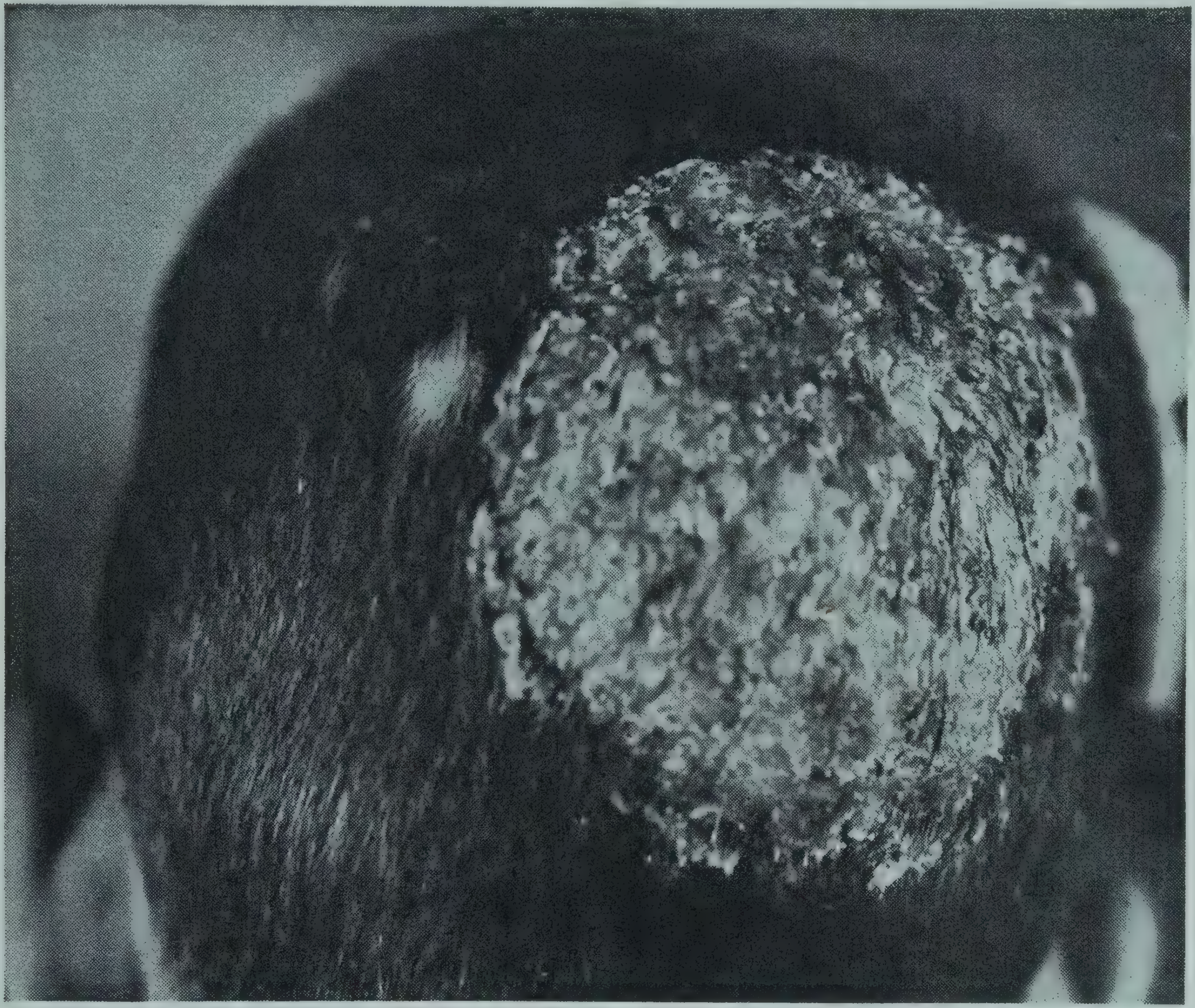

Fig. 1: Tinea of scalp

Genus	*Disease*
Microsporum	Microsporic tinea
Trichophyton	Trichophytosis
Epidermophyton	Epidermophytosis
Achorion	Favus

MICROSPORIC TINEA

The Microspora that cause tinea fall into two groups: (1) human origin, Microsporum audouini; and (2) animal origin, Microsporum lanosum. Each species has certain variants, some of which infect human beings.

Tinea capitis is caused by both Microsporum audouini and Microsporum lanosum. The latter also causes tinea of the beard and smooth skin. The diagnosis is made by microscopic examination of infected hairs in 40 per cent potassium hydroxide. Microsporum audouini surrounds the hair shaft in the form of spores from ten to twenty deep, which are neatly arranged. In the subcutaneous portion of the hair there may be wavy, branching mycelium, which runs toward the root and terminates in a fringe.

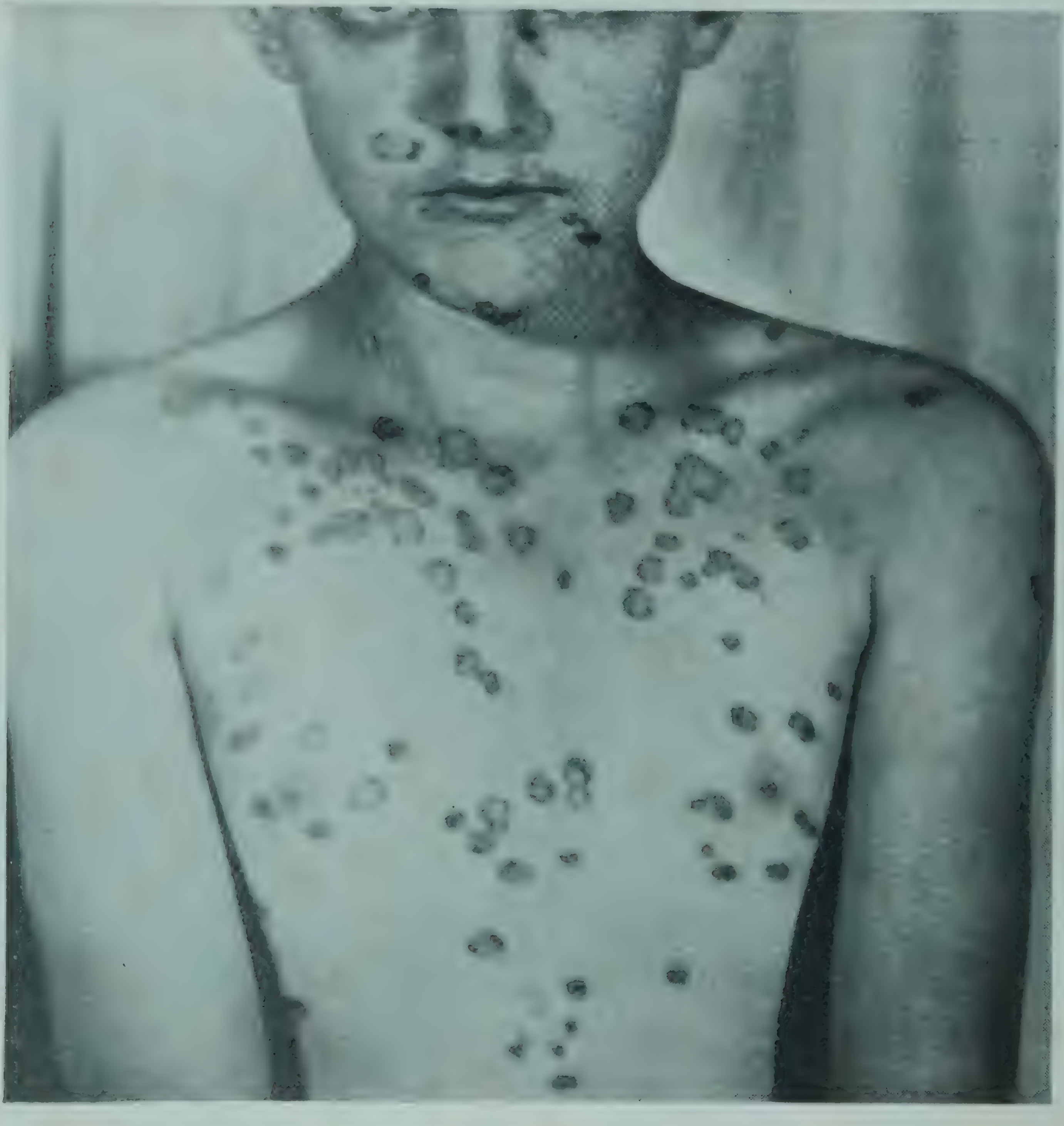

Fig. 2: Ringworm of chest.

The microscopic picture of Microsporum lanosum is similar to that of Microsporum audouini, except that the mycelium is much more profuse, and the mycelian filaments are not infrequently made up of coarse, oblong elements. Cats, dogs, and guinea pigs may be successfully inoculated with Microsporum lanosum.

TRICHOPHYTOSIS

The Trichophyta may produce ringworm of the scalp as well as a variety of lesions on the glabrous skin. Sabouraud classifies the Trichophyta as follows:

1. Trichopyton endothrix: This fungus is found only within the hair substance.
2. Trichophyton neoendothrix: In this group, some infected hairs also show a few filaments on the surface, but most are within.
3. Trichophyton ectothrix: This fungus, besides invading the substance of the hair, also proliferates actively on the hair surface.

Trichophyton Endothrix:

Trichophyton Crateriforme: This fungus causes ringworm of the scalp, but only rarely involves the smooth skin.

Trichophyton Acuminatum: Trichophyton acuminatum causes ringworm of the scalp; it involves the body or beard only rarely.

Trichophyton Violaceum: This parasite involves the scalp, beard, body, and nails.

Trichophyton Neoendothrix:

Trichophyton Cerebriforme: Trichophyton cerebriforme causes infection of the scalp, beard, and body in the form of tiny, follicular abscesses.

Trichophyton Ectothrix:

Gypseum Group: This group includes Trichophyton asteroides and certain related species. It causes ringworm of the scalp with typical kerion formation. It also causes tinea of the hands and feet. The beard and smooth skin are sometimes involved, and the infection manifests itself by pustules and vesicles.

Niveum Group: This group includes Trichophyton radians and one related species. It causes tinea of the smooth skin in the form of vesicles and pustules.

Downy Group: The downy group includes Trichophyton rosaceum and certain related species. It infects primarily the beard and nails.

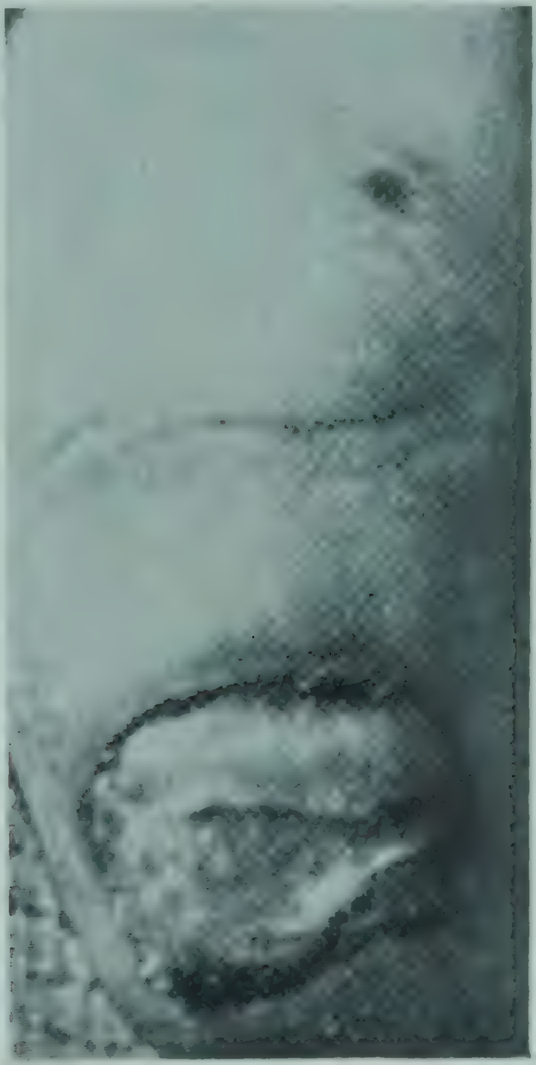

Fig. 3: Trichophytosis of finger nail.

Faviform Group: This group is especially parasitic for horses and cattle but is occasionally found in human beings. The species of this group are characterized by the fact that they will grow at first only at 98.6° F. (37° C.) and not at room temperature. Trichophyton ochraceum, Trichophyton album, and Trichophyton discoides are species of this group. All have been found in lesions of the trunk where they are likely to be impetiginous in character. Trichophyton ochraceum is found also in tinea of the beard.

EPIDERMOPHYTOSIS

The Epidermophyta resemble the Trichophyta, but Sabouraud separated them because they grow only in the superficial layers of the skin and do not invade hairs. Members of this genus are responsible for the ringworm condition of the hands and feet, which so many people have in one form or another. It is popularly called "athlete's foot."

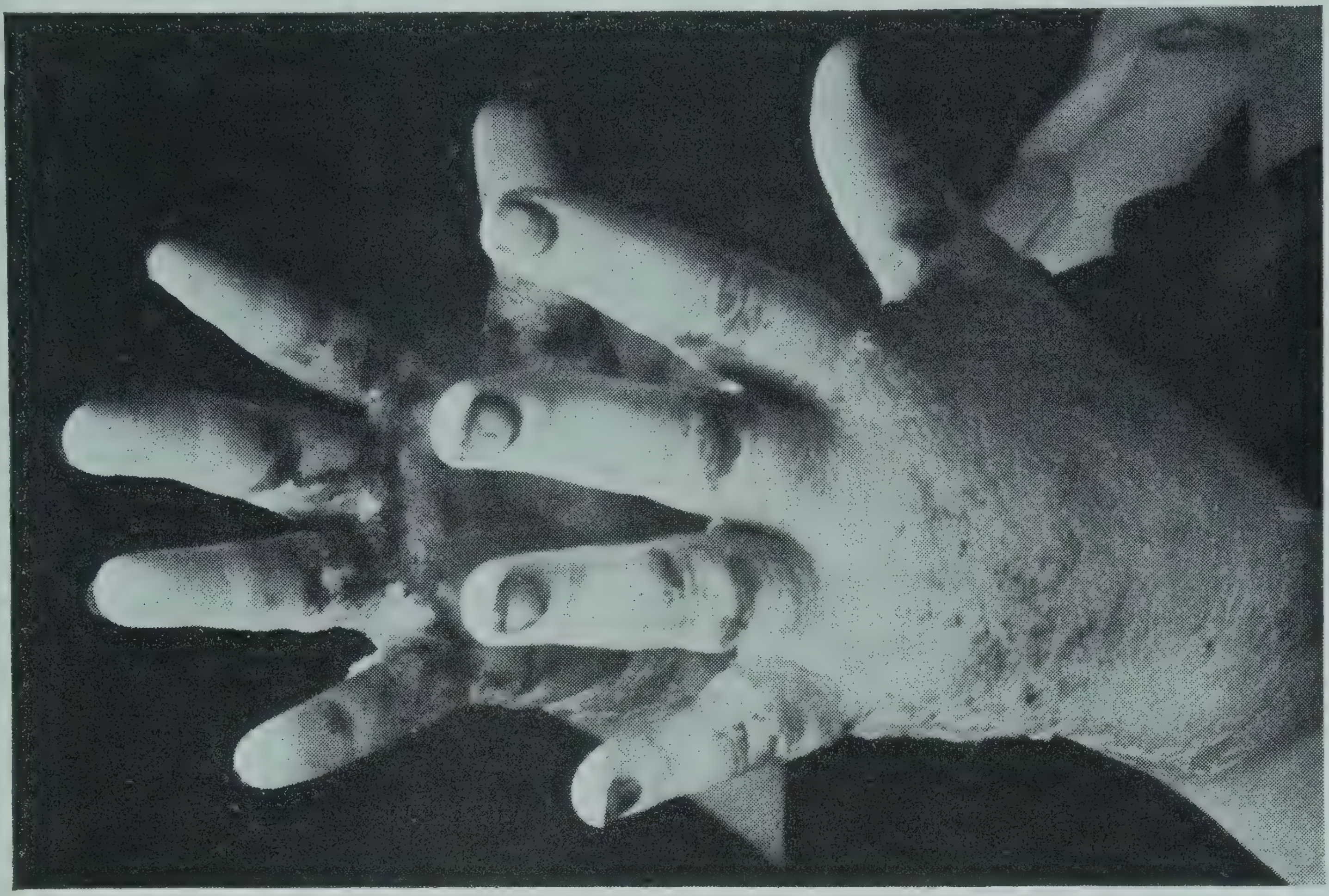

Fig. 4: Epidermophytosis.

Monomorphous Group:

Epidermophyton inguinale causes epidermophytosis of the groin and occasionally of the hands and feet.

Multiform Group:

Epidermophyton rubrum is characteristic of this group. It is found in the scales of tinea cruris and in the tops of vesicles of dyshidrosis. Tinea cruris is a form of eczema that involves the inner sides of the thighs, groin, and gluteal cleft, and occasionally the axilla. There are five related species that cause similar lesions.

FAVUS

Favus is caused by Achorion schoenleini and certain related species. Achorion schoenleini is essentially a Trichophyton of the endothrix type. Favus is a disease of the scalp, which usually occurs after puberty. The lesions are sulfur-colored, friable crusts in the form of cups or scutula pierced by hairs. Microscopic examination of involved hairs from scutula reveals the mycelium as wavy filaments made up of rectangular units parallel with the axis of the hair.

FUNGOUS INFECTIONS OF SKIN OR MUCOUS MEMBRANE WITH FREQUENT SYSTEMIC INVOLVEMENT

Moniliasis

Moniliasis is a fungous infection of the skin, mucous membranes, and internal organs, in which Candida fungi are found. Candida albicans may be present on the skin, in the mouth, and in the intestinal tract without causing any symptoms. It does cause active disease of various parts of the body surface, particularly the palms, nails, interdigital folds, axillas, knees, genital and anal regions, and external auditory canal. Of the mucous membranes, the mouth and vagina are most frequently involved. Thrush is not uncommon in the mouths of infants and undernourished children.

Systemic involvement particularly includes pulmonary and meningeal moniliasis.

Mycetoma

Mycetoma, or maduromycosis, is a chronic granulomatous disease usually limited to the distal portions of the lower extremities. It is fairly common in India and is found less frequently in Europe, Africa, and America. It is caused by various species of Actinomyces, various genera of fungi imperfecti, and Ascomycetes. Although the deeper tissues of the extremity may be involved, Mycetoma proper does not invade internal organs. Actinomyces may invade internal organs as well as the skin.

Microscopic examination serves to differentiate the two types. In the mycetomatous variety, the mycelia are segmented, walls are clearly defined, and there are numerous chlamydospores of various sizes and shapes. In the actinomycotic variety, the mycelian filaments are nonseptate, walls are not clearly defined in the mass of radial filaments, and chlamydospores are absent.

ACTINOMYCOSIS

This is the most frequent of the deep fungous diseases. It may occur as a local or constitutional disease caused by Actinomyces bovis or Streptothrix actinomyces. It appears to exist throughout the civilized world. The diagnosis is made by placing a sulfur granule from a lesion in 10 per cent potassium hydroxide and examining it under the microscope after crushing it with a coverslip. The microscopic picture reveals three zones: (1) a central zone composed of mycelian recticulum compactly arranged and studded with pigment granules; (2) an intermediate area, which is irregularly amorphous and deeply pigmented; and (3) an outer fringe, which is refractile, striated, and composed of mycelian hyphae that are nonseptate. They are very fine, arranged radially, and the extremities are expanded into club-like forms.

Actinomycosis also involves the tongue, lungs, intestines, female reproductive organs, urinary tract, central nervous system, and rarely the bones.

SPOROTRICHOSIS

Sporotrichosis is a subacute or chronic infectious disease caused by species of the genus Sporotrichum. The process is granulomatous in character and may be limited to the skin and subcutaneous tissues. Sometimes it involves internal viscera, bones, and the central nervous system.

Most infections in the United States are caused by Sporotrichum schenkii. Injuries to skin by thorns and briars, which are contaminated by the fungus, constitute the source of the disease. Internal infection may occur through eating contaminated raw vegetables or fruits.

The diagnosis may be made by direct examination or culture of pus or serosanguineous fluid from a granulomatous process. Direct examination in potassium-hydroxide preparations reveals oval or cigar-shaped bodies from 2 to 4 microns in length and from 1 to 3 microns in width, frequently located in large mononuclear cells. They resemble thick, short bacilli, and are finely granular and basophilic. Stained spores from pus are gram-negative, while preparations from artificial media are gram-positive. The mycelia and spores from artificial media are acid-fast.

BLASTOMYCOSIS

Blastomycosis is a cutaneous and systemic fungous disease caused by Blastomyces dermatitidis. In the United States, it occurs particularly around Chicago. The source of infection and method of transmission are still unsolved. It appears, however, that scratches and puncture wounds are routes of infection when the cause of the injury is a contaminated object.

The skin is involved primarily or secondarily in about 95 per cent of all recorded cases. The lungs are involved next in order of frequency. Every organ and important structure in the body is susceptible to infection by the Blastomycetes.

Microscopic examination of material from cutaneous abscesses reveals budding, yeast-like forms from 5 to 20 microns in diameter, containing granules and vacuoles, and surrounded by hyaline capsules. In tissue specimens with hematoxylin and eosin stain, the parasites may be seen within the giant cells or in granulation tissue as circular or oval bodies with a granular and occasionally vacuolated protoplasm. Budding is also seen in tissue preparations. In making a diagnosis of the systemic form of the disease, examination of the sputum, cerebrospinal fluid, or a biopsy specimen may reveal the organism.

COCCIDIOIDOMYCOSIS

This is a fungous infection, chiefly of the lungs, but sometimes involving the skin and other organs. It is caused by Coccidioides immitis, which was once limited to the plants of the San Joaquin Valley, California, but which has subsequently spread elsewhere. It may be localized and relatively benign, or systemic and rapidly fatal. It simulates other types of fungous infections, pulmonary tuberculosis, malignancy, and syphilis. Males are much more frequently infected than females.

Microscopic examination of pus from cutaneous lesions, sputum, spinal fluid, and tissue obtained from a biopsy may reveal the parasite. Morphologically, Coccidioides immitis is similar to the Blastomycetes. Coccidioides immitis is a sphere with a double-contoured capsule, and it varies in diameter from 5 to 60 microns. In tissues, the organisms are seen within giant cells or situated loosely in the granulation tissue. When grown on artificial media, it appears as an intricate mycelian network, with long, branched, septate hyphae, and chlamydospores and conidia in old cultures.

PRIMARY SYSTEMIC INFECTIONS

Cryptococcosis

Cryptococcosis, or torulosis, is caused by Cryptococcus neoformans, or Torula histolytica, and it usually manifests itself in the form of a meningitis. Cutaneous lesions are rarely secondary to meningitis. Infection apparently takes place through the respiratory tract. It is found as a saprophyte on the skin, in the throat, and in the intestinal tract.

Cryptococcus neoformans, unstained, may be mistaken for lymphocytes or erythrocytes in the spinal fluid. They are oval or round cells, which measure from 3 to 15 microns in diameter and are enclosed by definite cell walls that stain with methylene blue. They are gram-positive when young. Their only method of reproduction is by budding.

Aspergillosis

Aspergillosis is a rare fungous infection of the lungs and skin, which is caused by Aspergillus fumigatus, Aspergillus niger, or other species of Aspergillus.

The diagnosis is made by examining microscopically and culturing sputum or pus. The parasite is characterized by a stalk and a spore-bearing head. The color, shape, size, and arrangement of parts vary in different species and constitute distinguishing characteristics for some of the species of Aspergillus.

Histoplasmosis

This is a rare, fatal, systemic disease caused by Histoplasma capsulatum, which invades the reticuloendothelial system. The diagnosis is made by finding the parasite in the cytoplasm of the large mononuclear cells of the blood or in smears of the sternal bone marrow. When stained with Wright's stain, they appear as bluish or bluish-red, oval cells varying from 2 to 4 microns in diameter and surrounded by a clear, capsule-like substance. Blood inoculated intraperitoneally into guinea pigs produces a chronic, afebrile, systemic disease with changes similar to those in human beings. According to Van Pernis and co-workers,[2] the intracutaneous-skin test with filtrates of sterile-broth cultures of Histoplasma capsulatum appears to be a helpful diagnostic procedure.

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CHAPTER 16

Skin Tests

INTRODUCTION

Skin tests are employed to determine the sensitivity of persons to certain exciting agents and to aid in the diagnosis of some of the diseases caused by bacteria, fungi, parasites, and viruses. These tests are used to determine specifically what agent or agents cause pollen, and drug allergies. Skin tests are also of value in determining the status of immunity to certain diseases, particularly diphtheria and scarlet fever.

The exciting agents may gain access to the body in the following ways:

1. Respiratory tract (air-borne pollens and dusts)
2. Intestinal tract (ingestion of food and fluids)
3. Skin (contact with ivy, oak, sumac, drugs, and so on)
4. Skin (injections of sera, drugs, insect venom, and so on)
5. Infection by bacteria
6. Infection by fungi
7. Infection by parasites
8. Infection by viruses

TYPES

Scratch Test:

A superficial abrasion not over 5 mm. (1/5 inch) long is made on the sterilized skin of the flexor surface of the forearm. Sometimes, other sites, such as the lateral surface of the upper arm or the back, are used. If many tests are to be carried out, the scratches should be made at one time and should be about 1 cm. (⅖ inch) apart. The test materials are applied to the scratches with the appropriate diluent. Only the diluent is applied to a control scratch. After the development of a positive test or after twenty minutes, the materials are wiped off and the readings are made. Reactions are read as follows:

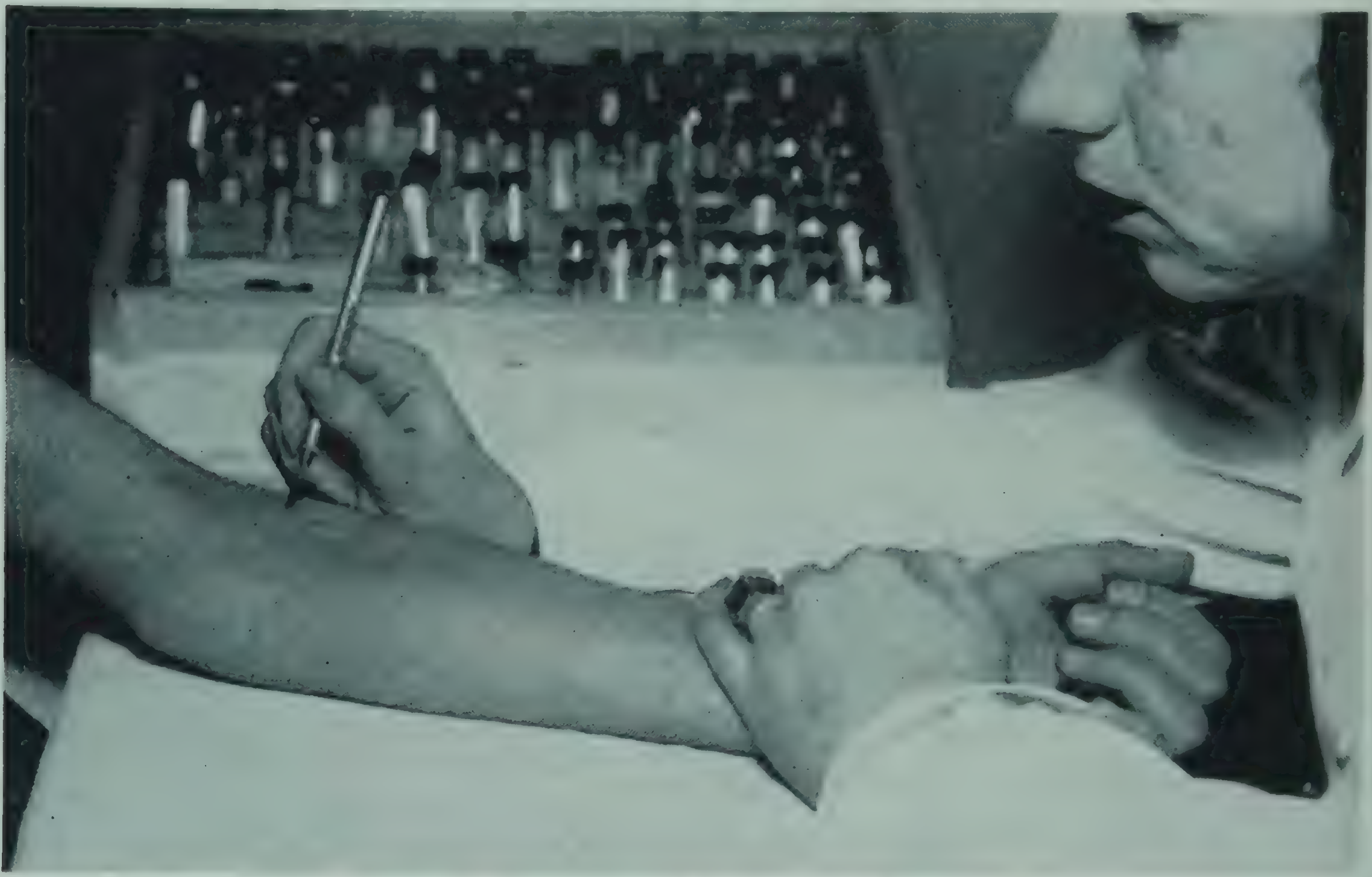

Fig. 1: Skin testing for allergy.

Negative: The wheal is not larger than the control. The areola is absent or not more marked than the control.

Slight: The wheal is about 0.5 cm. (⅕ inch) in diameter. The areola is slight.

Moderate: The wheal is between 0.5 and 1 cm. (⅕ and ⅖ inch) in diameter, and no pseudopodia are present. The areola is moderate.

Marked: The wheal is more than 1 cm. (⅖ inch) in diameter and has pseudopodia. The areola is marked.

Intracutaneous Test:

The agents are injected intracutaneously in the sterilized skin of the lateral surface of the upper arm. Not over 0.02 cc. should be used in each injection, and it is advisable not to do more than twelve tests at one time. With this type of test, it is important to watch carefully for constitutional reactions. After five to fifteen minutes, the reactions are read according to the criteria used for the scratch test.

Patch Test:

The skin is cleaned, and the material is applied on a piece of cloth about 1 cm. (⅖ inch) square. This is covered with rubber or cellophane and is kept in place with adhesive tape for twenty-four hours. The flexor surface of the forearm or the back may be used. If the reaction is negative at the end of twenty-four hours, it should be observed daily for four days and periodically for two weeks because some agents cause a delayed reaction.

A positive reaction consists of a red area with tiny vesicles. A positive reaction should not be confused with reaction to adhesive tape around the periphery of the rubber covering.

FOR PRIMARY ALLERGENIC DISEASES

Hypersensitiveness to certain air-borne, ingested, contacted, and injected agents is called "allergy." This term was coined by Von Pirquet and means "changed, or altered, reactivity." The term "anaphylaxis" which means "without protection" is reserved for those manifestations of hypersensitiveness in the lower animals in which an antibody mechanism has been demonstrated.

Certain human beings have natural or acquired hypersensitiveness to various agents. This is manifested chiefly in asthma, rhinitis, urticaria, various types of eczema, and migraine. Tests for inhalants, foods, and contactants can be carried out with the specific agents by the scratch or intracutaneous tests.

Great care must be used in interpreting the results of skin tests. A positive reaction to a given agent does not necessarily mean that that particular agent is the cause of the clinical condition. A clinical trial of treatment must be carried out by eliminating the agent from the diet or from contact to see if it is the cause of the hypersensitiveness. False-negative skin tests may mean that the skin itself is not hypersensitive to the agent, but the patient may still have a systemic sensitiveness or allergy. Some persons have a marked reaction to the scratches alone, and this may be a reaction to the histamine of the skin. In any event, it makes it difficult to interpret the tests.

Serum Allergy:

Certain persons are allergic to antitoxin and antibacterial serum prepared from horses. They are allergic to the horse serum rather than to the antibody content of the serum. The symptoms include urticaria, fever, glandular swelling, edema, and joint pains. The outcome may be fatal unless precautions are taken to give small amounts of the serum over a prolonged period.

In cases of natural allergy, a scratch test is satisfactory. If acquired allergy from a previous injection of antitoxin is suspected, an intracutaneous test is preferable. This consists of the injection of 0.02 cc. of a 1:10 dilution of normal horse serum.

FOR BACTERIAL INFECTIONS

The cell substance of bacteria is antigenic and may act as a sensitizing agent. Hypersensitiveness to bacteria may be acquired in the course of an acute or chronic infection, or during a clinically unrecognized infection. In certain diseases, the skin test is reliable enough to be of diagnostic aid.

Tuberculosis:

The scratch test (Pirquet's reaction), the intracutaneous-tuberculin test (Mendel's test), or the patch test (Vollmer's test), may be used. Old tuberculin (OT) or purified protein derivatives (PPD) may be used. The intracutaneous-tuberculin test with purified protein derivative is considered the best. A positive reaction is characterized by an area of erythema and swelling 5 mm. (⅕ inch) or more in diameter after forty-eight hours. Necrosis may be present in marked reactions. The test is positive in clinically active and inactive tuberculosis. Since the majority of adults have had a subclinical infection at some sime, the test is of little value in this group. The test is negative in nontuberculous persons, very early in the disease, during the terminal stage of pulmonary tuberculosis, in the poorly nourished, and in the course of some acute infectious diseases.

This test is of most value in children up to five years of age. A negative tuberculin test is significant at any age. In reading all tests, they should be compared with the control in order to make sure that the reaction is due to the tuberculin alone.

Undulant Fever:

An intracutaneous test is used in addition to the agglutination test and opsonocytophagic index in the diagnosis of undulant fever (brucellosis). The material used is 0.1 cc. of Brucella protein designated as "abortin," or "brucellergen." The test is read after forty-eight hours. A positive reaction consists of an area of erythema, induration, and edema. The patient should be warned that necrosis may take place.

A positive agglutination test, a high opsonocytophagic index, and a positive skin test together indicate infection. A certain proportion of positive skin tests occurs in clinically inactive cases.

A negative skin test usually means that the disease is not present, particularly if the agglutination test and opsonocytophagic index are negative. The test may be negative, however, in patients who have Brucella abortus in blood cultures because the skin is not sensitized.

Chancroid:

Bacteriological examinations of material from a lesion for Hemophilus ducreyi is more conclusive than the skin test. The test[1] is performed by the intracutaneous injection of 0.1 cc. of a killed culture suspended in normal saline. The test is read after seventy-two hours. The test does not become positive until from eight to fifteen days after the appearance of the lesion. A negative test is of most value because sensitivity persists for years after the lesion has healed.

As indicated above, bacteriological examination is preferable, but a negative skin test in the presence of a long-standing genital lesion rules out chancroid.

Tularemia:

The intradermal injection of a killed suspension of Pasturella tularensis gives a positive test by the fourth day of the disease. The agglutination test and guinea-pig inoculation of material from a lesion are preferable, however, in making a diagnosis.

A negative skin test does not rule out the disease, and a test may be positive without active disease, since the allergy persists for years after the illness.

Glanders:

The mallein test is comparable to the tuberculin test, since mallein is the concentrated glycerol broth in which the glanders bacillus Malleomyces mallei has grown. Mallein is injected subcutaneously, and the size of the dose varies with the concentration of the material. The mallein reaction consists in a rise of temperature with a hot, painful swelling at the site of inoculation. The swelling increases in size up to from twenty-nine to thirty-six hours, persists for about a week, and gradually disappears. Veterinarians agree regarding the diagnostic value and specificity of mallein, but it has not been used in human beings sufficiently to evaluate its efficiency.

Diphtheria:

In 1913, Schick introduced an intradermal test, by which it is possible to select those who are immune to diphtheria because of natural or acquired immunity. In an epidemic of diphtheria, it is of value in determining those who require prophylactic antitoxin. It is not used in the diagnosis of the active disease.

The test is carried out by injecting 0.1 cc. of the diluted toxin intradermally into the skin of the flexor surface of the forearm. In positive reactions, a red, raised area from 1 to 2 cm. ($\frac{2}{5}$ to $\frac{4}{5}$ inch) in diameter develops in from twenty-four to thirty-six hours and reaches its height about the fourth day. A positive reaction indicates susceptibility to diphtheria, while a negative reaction indicates immunity. The number of positive reactions is greater in children than in adults.

Unfortunately, a few false-positive reactions, or pseudoreactions, occur. These are the result of allergy to a product in the toxin. They may begin to develop as early as six hours after injection, reach the height in thirty-six hours, and disappear by the fourth day. It is safer, therefore, to read the Schick test between the fourth and sixth days. Pseudoreactions have a central, dusky-red area 1 cm. (⅖ inch) in diameter or slightly larger. This is surrounded by a pale red areola.

Schick material may be purchased ready for use from biological supply houses.

Scarlet Fever:

George F. Dick and Gladys H. Dick devised a test for immunity to scarlet fever. It is carried out by the intradermal injection of 0.1 cc. of a 1:1000 dilution of the Berkefeld filtrate of a culture of a streptococcus that has been isolated from a case of scarlet fever and can produce the disease in human beings. This filtrate can be purchased from biological supply houses.

Reactions should be read between eighteen and twenty-four hours after the injection. A positive reaction indicates absence of antitoxin, which means that the person is susceptible to scarlet fever. A negative reaction indicates immunity to the disease, although a few may be susceptible to a local (throat) infection with a hemolytic streptococcus.

A positive reaction varies from a pink area not raised, to a deep red, raised, swollen area from 3 to 5 cm. (1⅕ to 2 inches) in diameter. False-positive reactions from infection of the skin may result from careless handling of the material. Pseudoreactions from allergic sensitivity to the material are rare.

FOR FUNGOUS INFECTIONS

Cutaneous:

Cutaneous fungi vary in their ability to sensitize the skin. Intracutaneous tests with trichophytin, however, have been helpful in the diagnosis of certain fungous infections of the skin. In general, however, microscopic examination and culture of material from the lesion are preferable.

Coccidioidomycosis:

The intradermal injection of coccidioidin, which is prepared from cultures of Coccidioides immitis, is claimed by Dickson[2] to be of value in the diagnosis of coccidioidomycosis. Previous pulmonary infection must be ruled out if the test is used to diagnose the cutaneous form of the disease because sensitization persists for a long period of time.

Histoplasmosis:

According to Van Pernis and associates,[3] the intradermal injection of histoplasmin, prepared from filtrates of broth cultures of Histoplasma capsulatum, gave positive reactions in a case of systemic histoplasmosis. They injected 0.1 cc. of the undiluted filtrate and various dilutions of the filtrate (1: 10, 1: 100, and 1: 1000) and noted immediate and delayed reactions.

Sporotrichosis:

Sporotrichin, which is prepared from the filtrate of cultures of Sporotrichum schenckii, is used in the intradermal test for sporotrichosis. A negative test is particularly valuable in ruling out the presence of the disease, but false-positive reactions may occur in the presence of certain other fungous infections.

FOR PARASITIC INFECTIONS

Trichinosis:

Material for precipitin and skin tests is prepared by making extracts of larvae that are digested out of infected muscle,[4] particularly diaphragmatic muscle. The intradermal test is positive in almost 100 per cent of active cases. It becomes positive after about two weeks and remains positive for several years. An immediate reaction within twenty minutes, characterized by a wheal with an areola of hyperemia, develops during the second week of the active disease. Earlier, the reaction may not develop for twenty-four hours.

The precipitin test and muscle biopsy are sometimes necessary to establish a diagnosis.

Hydatid Cyst:

The intradermal injection of 0.01 cc. of diluted (1: 10,000) hydatid-cyst fluid is employed in the diagnosis of hydatid cyst. I used a saline extract of scolices, which proved to be equally as sensitive as hydatid-cyst fluid.

A positive reaction takes place in from fifteen to twenty minutes and is characterized by a pink to red wheal with or without pseudopodia. The presence of hydatid cyst cannot be entirely excluded by a negative test.

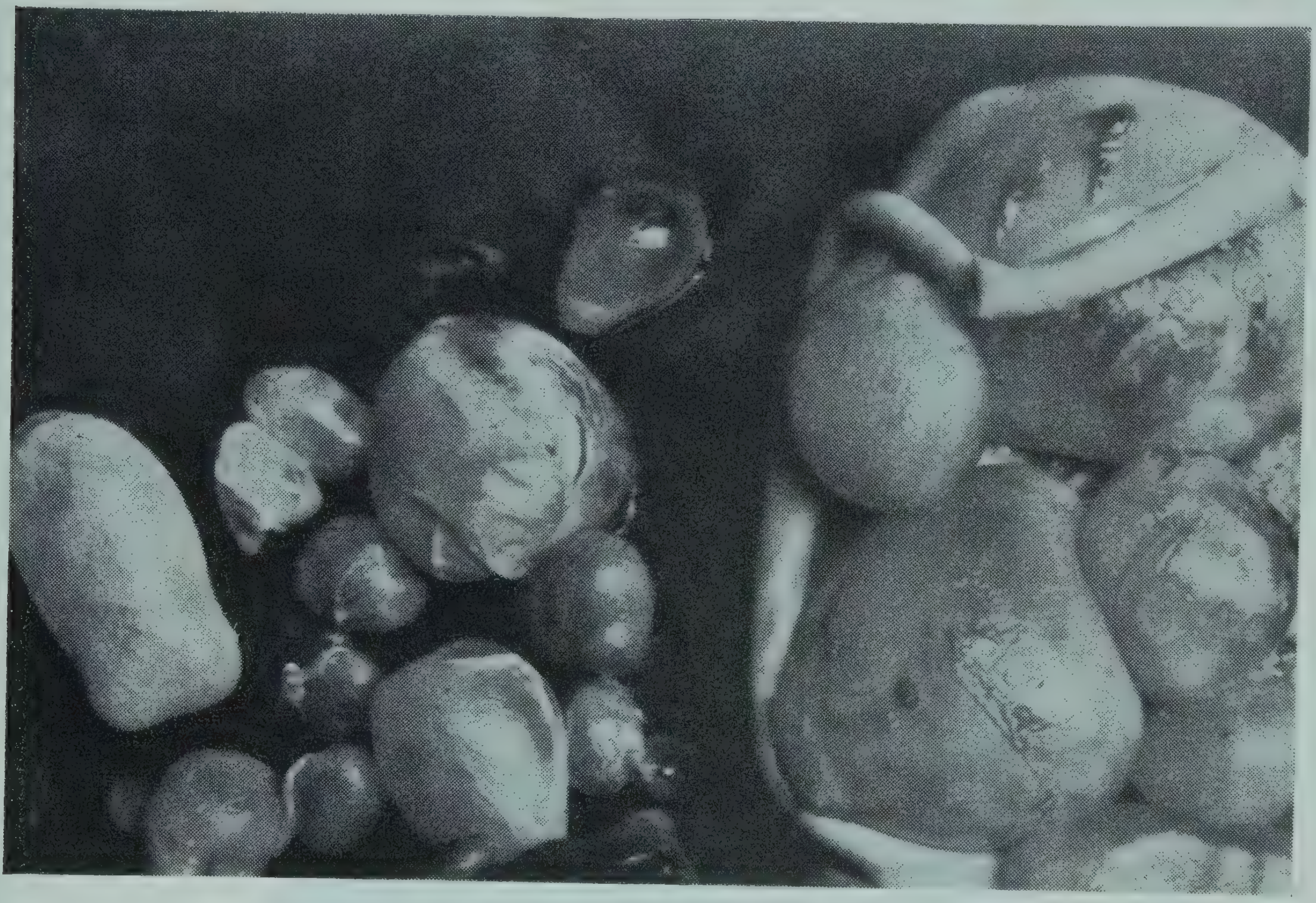

Fig. 2: Hydatid cyst showing chitinous membrane and daughter cysts.

Filariasis:

About 90 per cent of persons with Wuchereria-bancrofti infestation have a positive intradermal test with material prepared from filarial worms of lower animals, particularly Dirofilaria immitis.[5] The reaction usually occurs within from fifteen to twenty minutes, but delayed reactions do occur. It is advisable to read negative reactions again at the end of twenty-four hours.

Schistosomiasis:

An intradermal test with cercarial antigen prepared from snails infested with Schistosoma mansoni or Schistosoma japonicum is positive in a large proportion of persons with schistosomiasis. The test remains positive long after the disease is cured, and a few false-positive reactions occur because of sensitivity to snail protein.

Leishmaniasis:

Phenolized saline suspensions of dead Leishmania tropica are used for intradermal tests. They give position reactions only in cutaneous leishmaniasis but may also give weakly positive reactions in about 10 per cent of persons free of the disease.

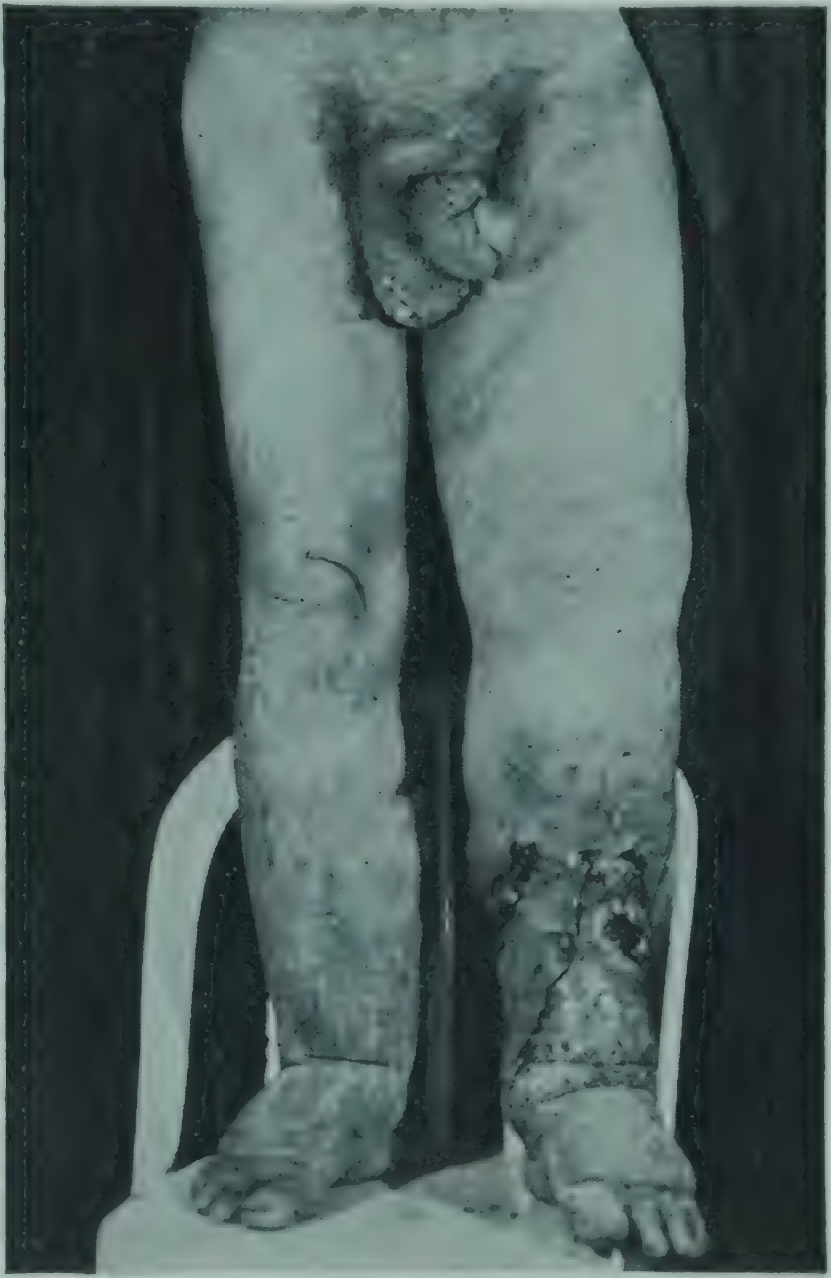

Fig. 3: Elephantiasis with ulcers of left ankle (Wuchereria bancrofti).

FOR VIRUS INFECTIONS

Lymphopathia Venereum:

The Frei test for lymphopathia venereum consists of an intradermal test with pus from a lesion,[6] infected mouse-brain suspension,[7] or material from an infected chick embryo.[8] This material, together with a control, is supplied commercially. The test is read in forty-eight hours. A positive test is characterized by an infiltrated inflammatory area with a small, well-defined papule.

Mumps:

This test is usually not necessary to make a diagnosis, but mumps does develop altered skin sensitivity to a suspension of infected, monkey, parotid glands[9] or material from an infected chick embryo.[10,11] The positive test reaches its height in forty-eight hours and is characterized by an indurated inflammatory area. A positive reaction may be caused by past, as well as present, infection, and so the test must be interpreted in the light of the clinical picture.

Influenza:

Beveridge and Burnet[12] have shown that infection with influenza-A or -B viruses results in the development of dermal hypersensitivity to the specific virus or some product of this virus. No practical use has been made of this test as yet for routine diagnostic work. Neutralization tests, complement-fixation tests, and hemagglutinin-inhibition tests, in addition to the skin tests, are useful in diagnosing this disease. The last three tests can differentiate between types A and B and other influenza viruses.

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CHAPTER 17

Toxicologic Examinations

In certain cases of poisoning, it is helpful to have qualitative or quantitative examination of body fluids or excreta. When the person is killed by a poison, appropriate body fluids and tissues are subjected to chemical examination. In instances where the person survives the poison, examination of the blood, gastric contents, urine, or feces may be helpful in determining the nature and the amount of the poison.

Gonzales and associates[1] classify poisons as follows:

1. Gaseous poisons (poisons present in the gaseous state)
2. Inorganic poisons
    a. Corrosive (poisons characterized principally by an intense and destructive local action; a few organic corrosives are included in this group for the sake of completeness)
    b. Metallic poisons and salts (these chemicals are protoplasmic irritants, but their chief action is the deleterious effect produced after absorption into the system)
3. Organic Poisons
    a. Volatile poisons (volatile liquids or easily sublimated solids, many of which are irritants; their chief action occurs after absorption)

   b. Alkaloidal poisons (these substances are toxic principles of plants that have a characteristic action on some part of the central nervous system; they are a well-defined group)
   c. Nonalkaloidal poisons (a conglomerate collection of the other organic, toxic substances, nonvolatile and nonalkaloidal)
4. Miscellaneous poisons (associated with botulism, food poisoning, mushroom poisoning, and snake-venom poisoning)

Only those poisons that the clinician is likely to encounter in the living person are considered here.

GASEOUS POISONS

Carbon Monoxide:

Carbon monoxide in its pure form is a colorless, odorless, and tasteless gas. It is formed in any process in which there is incomplete combustion of carbon. It is produced in furnaces, stoves, and gasoline engines, during conflagrations and bomb explosions, and in the combustion of natural gas, coal, coke, and charcoal. Illuminating gas in the household and automobile engines constitute the most common sources of poisoning.

In cases of poisoning with carbon monoxide, the gas is inhaled into the lungs where it comes in contact with the hemoglobin of the red cells in the pulmonary capillaries. Carbon monoxide has an affinity for hemoglobin two hundred times greater than that of oxygen. It forms carboxyhemoglobin, which is more stable than oxyhemoglobin. If enough of the oxyhemoglobin is replaced by carboxyhemoglobin, death will take place as a result of asphyxia.

Persons vary in their susceptibility to carbon monoxide. Those who are subjected constantly to the gas, for example in unventilated garages, may develop a concentration of from 5 to 6 per cent in the blood without demonstrable symptoms. In fatal cases, the concentration may vary from 40 to 70 per cent in the blood. The concentration of the gas, the length of exposure, and rate of respiration are important factors in determining the outcome of the patient.

When the person is removed to fresh air, the blood starts to free itself of carbon monoxide. Complete elimination of the gas takes place in about four to twenty-four hours, depending on the concentration. Blood for examination for carbon monoxide, therefore, should be taken as soon as possible after exposure. From an arm vein, a few cubic centimeters of blood, placed under oil in a stoppered container, is satisfactory. It should be kept in the dark and analyzed as soon as possible. Both chemical and spectroscopic tests are used to detect the presence of carbon monoxide, but unfortunately they require a saturation of 10 per cent or more.

If laboratory facilities are not available for chemical or spectroscopic examination, a simple test may be carried out by warming the blood on a water bath. A few cubic centimeters of blood is placed in a test tube and heated in a water bath. A normal control is treated in the same way. A brick-red coagulum forms in the tube containing carboxyhemoglobin, while normal blood forms a brown-black coagulum.

Other gases, such as hydrogen sulfide, sulfur dioxide, nitrous oxide, nitric oxide, fluorine, bromide, chlorine, phosgene, and chloromethylchloroformate, do not ordinarily concern the clinician.

INORGANIC POISONS

The corrosive acids and alkalies do not lend themselves to laboratory analysis after ingestion.

Arsenic:

Various arsenic compounds are used, such as skin lotions, hair dyes, and medicine. Arsenic also occurs in water and sea food, and on certain fruits that have been sprayed with arsenical compounds. There is likely to be, therefore, a small amount of arsenic in the urine. According to Meyers and Cornwall,[2] there may be up to 0.15 mg. in a twenty-four-hour volume of urine or up to 0.4 mg. per 100 gm. of dried urine. Examination of the blood, feces, and hair may be carried out, but they are less satisfactory. In cases of recent ingestion of an arsenic compound, analysis of the gastric contents or vomitus will reveal the presence of the drug.

Paris green (copper acetoarsenite) and white arsenic (arsenic trioxide) are more frequently used with suicidal intent. They are more toxic than the organic compounds such as neoarsphenamine. Nausea, vomiting, and abdominal cramps should arouse the suspicion of the physician so that he may have the vomitus or gastric contents analyzed for poisons. The lethal dose is difficult to estimate.

Lead:

Poisoning by lead compounds is more likely to be a result of industrial exposure than of ingestion with suicidal intent. Ingestion of lead may also occur accidentally, as, for example, in those communities where lead pipes are used to carry water. The fatal dose is difficult to estimate, because 10 gm. of lead acetate may be lethal for some persons while 30 gm. may not be lethal for others.

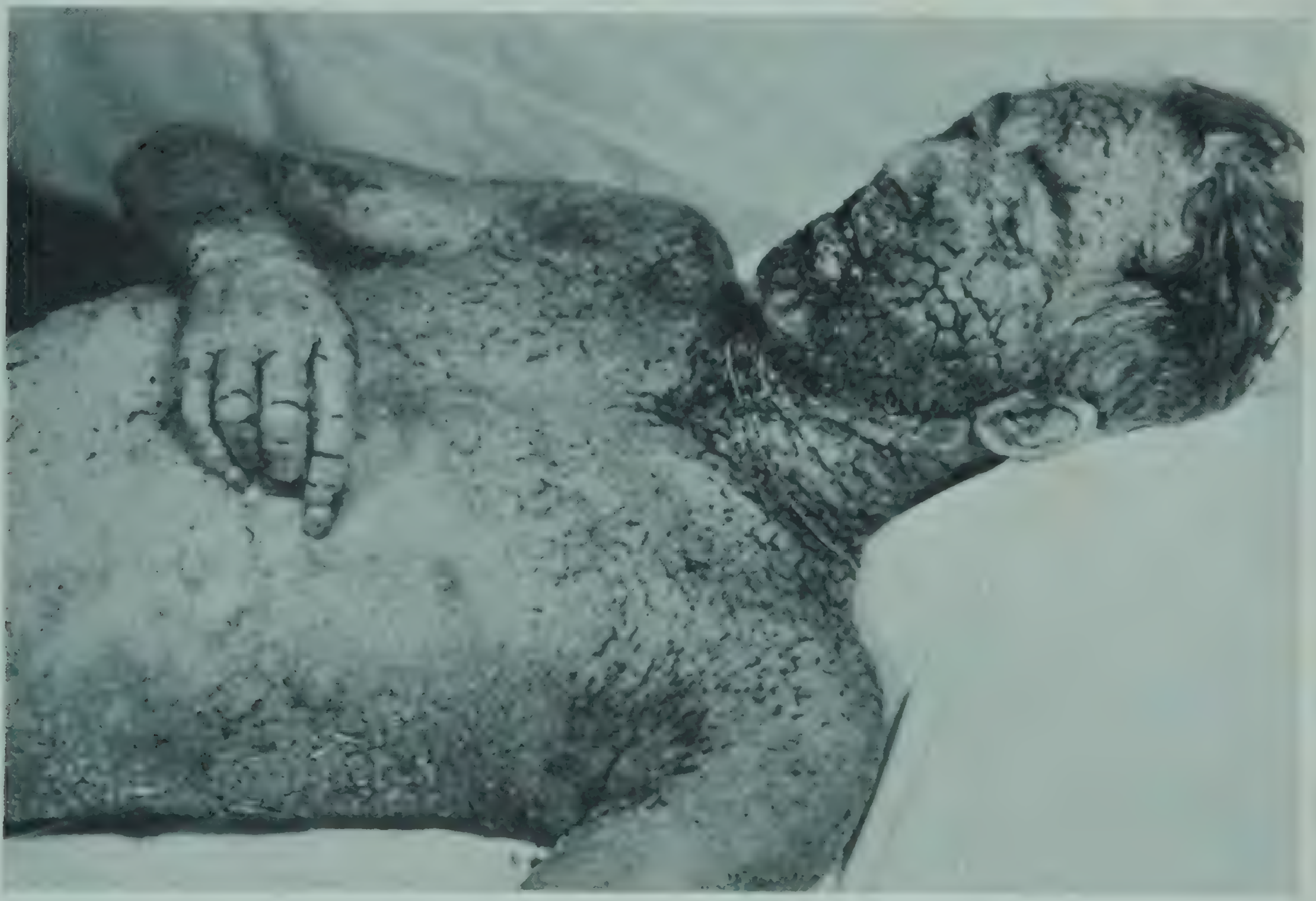

Fig. 1: Dermatitis from arsenic poisoning.

There is apparently more universal exposure to lead than was previously believed because the majority of people excrete a small amount of lead in the urine. They do not have any symptoms of intoxication. Kehoe, Thamann, and Cholak[3] analyzed the urine from a series of persons who had no particular exposure to lead and found from 0.021 to 0.038 mg. per liter. Other reports indicate that normal persons may have as high as from 0.080 to 0.10 mg. per liter. According to Kehoe and associates,[3] in those persons exposed to lead, a urinary excretion not exceeding 0.052 mg. per liter is regarded as safe, 0.097 mg. per liter approaches the toxic level, 0.175 mg. per liter is dangerous, and 0.352 mg. per liter is indicative of very hazardous exposure.

The normal range for lead in feces is 0.240 mg. per day;[4] for blood, 0.060 mg. per 100 cc.;[5] and for cerebrospinal fluid, 0.001 mg. per 100 cc.[4]

Examination of the twenty-four-hour specimen of urine is the preferable method of making a diagnosis of exposure to lead. The urine must be collected in a lead-free container, and the analysis must be carried out with lead-free reagents. If the physician carefully correlates the urinary lead excretion with the history, physical findings, and blood-smear examination for stippled cells, then a diagnosis of lead poisoning can be properly evaluated. In evaluating such a case, due consideration must be given to the co-called "normal" lead excretion.

Five per cent or more of stippled cells is found more frequently in lead poisoning than in any other condition.

Mercury:

Metallic mercury is not poisonous, but it tends to form toxic compounds. The mercuric salts are very toxic, while the mercurous salts are quite insoluble and much less toxic. The poisonous mercuric salts are mercuric chloride (corrosive sublimate), mercurammonium chloride, red mercuric iodide, yellow mercuric oxide, mercuric salicylate, and mercuric cyanide. The toxic mercurous compounds are mercurous chloride (calomel), yellow mercurous iodide, and the black oxide. Calomel is used as a cathartic and is usually harmless; but under some conditions, it may be changed in the body to mercuric chloride and cause poisoning.

Organic mercury compounds are used as diuretics, germicides, and therapeutic agents in the treatment of syphilis. Among these are mercuric benzoate, mercuric oxycyanide, merbromin, mersalyl, and mercurosal. Care must be exercised when using these compounds.

Mercuric chloride (corrosive sublimate) causes most cases of mercury poisoning. It is ingested with suicidal intent or a tablet is taken by mistake for some other drug. Fatal poisoning has also resulted from using it as a vaginal douche to prevent conception. Mercury is absorbed from the intestinal tract, respiratory tract, skin, and vagina. After it enters the bloodstream, it is taken up by the liver, spleen, and kidneys. It is excreted in the urine within fifteen hours after ingestion and in the feces in thirty to forty hours. No mercury is found in the urine or feces in the absence of exposure to the compounds of the metal.

Copper:

Copper poisoning occurs as a result of ingestion of one of the salts by accident or with suicidal intent. The salts of particular significance are copper sulfate (blue vitriol), copper subacetate (virdigris), and copper acetoarsenate (Paris green). Copper sulfate is most commonly encountered as a suicidal poison; and occasionally, cases of poisoning result from introduction of the solution into the vagina or application to scalp or skin for ringworm.

Copper is present in foods, and there is a daily intake of from 2.0 to 2.5 mg. per day by adults and from 0.07 to 0.12 mg. by children. Most of the copper is eliminated in the feces; however, an average of 0.3 mg. per liter is excreted in the urine. In cases of ingestion of a copper compound, examination of the gastric contents and later the urine may be helpful.

ORGANIC POISONS

Methyl Alcohol:

Methyl-alcohol poisoning occurs most frequently by drinking it as a substitute for ethyl-alcohol beverages. After it is absorbed, it is oxidized slowly to formaldehyde and formic acid. These products are retained in the body for a few days and are excreted in the urine or broken up into carbon dioxide and water. Quantitative examination of the urine for formic acid is helpful if there is an excessive excretion. According to Autenrieth,[6] normal persons eliminate 251 mg. of formic acid in the urine in twenty-four hours. This must be considered in evaluating the results.

The fatal dose varies in different persons. Death has followed the ingestion of from 30 to 60 cc. of methyl alcohol, and blindness has been caused by 15 cc. Death may occur in twenty-four hours, or the person may live as long as four days.

Ethyl Alcohol:

Poisoning by ethyl alcohol is usually accidental. It occurs when a person drinks to excess and absorbs more than the system can assimilate and excrete. The state of intoxication, however, is more frequently encountered by the clinician, and for this analysis of the blood has been helpful. The serum or plasma should be examined as soon as possible, but it may be kept in a refrigerator up to five days; or if 10 mg. of sodium fluoride per cc. of blood is added, it may be kept for several weeks.

The following results are given by Turner:[7]

State of Intoxication	*Blood Alcohol Level in Per Cent*
No evidence of intoxication	0.10 to 0.20
Slight intoxication (stands and walks, but talks incoherently)	0.21 to 0.30
Marked intoxication (falls frequently)	0.31 to 0.40
Alcoholic stupor (attempted answers and movements)	0.41 to 0.50
Alcoholic coma	0.51 or above

The fatal dose is difficult to ascertain, but a level of from 0.70 to 1.0 per cent in the blood is considered sufficient to cause death.

Barbital:

Barbital and barbituric-acid derivatives are used as hypnotics. Many cases of fatal poisoning, both accidental and suicidal, occur from an overdose of one of these compounds. About 50 to 90 per cent of barbital is eliminated by the kidneys, while the remainder is oxidized in the body. Cases of agranulocytosis have been ascribed to the barbiturates.

Other organic compounds that cause poisoning when taken in large amounts are morphine, heroin, codeine, acetanilid, acetphenetidin, strychnine, digitalis, formaldehyde, isopropyl alcohol, fusel oil (amyl alcohol), pyrogallol, and iodoform. Chemical examination of the urine may be carried out when poisoning is suspected by any of these compounds.

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PART 2

Diseases with Associated Laboratory Findings

CHAPTER 18

The Blood and Hematopoietic Organs

THE ANEMIAS

This chapter concerns itself primarily with those diseases that result from the abnormalities of the erythrocytic series of blood cells. In most diseases, the process is not one that originates in the blood; but it is reflected in the blood, as for instance hemorrhage from a peptic ulcer, benzene poisoning, or a deficiency disease.

CLASSIFICATION

The ideal classification is based upon etiology. However, our knowledge of the etiology of anemias is incomplete at this time, and hus present classifications are not entirely satisfactory. The following classification is modified from Kracke:[1]

I. Anemias due to blood loss
    A. Extravascular loss
        1. Acute hemorrhage (bone marrow, hyperplastic)
        2. Chronic hemorrhage (bone marrow, hypoplastic; blood picture, hypochromic)

- B. Intravascular destruction and hemolysis (hemolytic anemias)
  - 1. Symptomatic hemolytic anemias
    - *a.* In bacterial and parasitic infections (caused by Clostridium or hemolytic streptococcus, or such infections as Oroya fever or malaria)
    - *b.* After incompatible blood transfusion
    - *c.* Caused by drugs and chemicals (lead poisoning, sulfonamides in certain persons, phosphorus, organic arsenic compounds, pyridine, phenylhydrazine, acetylphenylhydrazine, potassium chlorate, trinitrotoluene, dinitrobenzene, and acetanilid)
    - *d.* From animal poisons (venoms)
    - *e.* From vegetable poisons (favism from ingestion of Vicia fave)
  - 2. Idiopathic hemolytic anemias
    - *a.* Congenital hemolytic anemia
    - *b.* Acquired hemolytic anemia
    - *c.* Acute hemolytic anemia of Lederer
    - *d.* Erythroblastic anemia
      - (1) Erythroblastosis foetalis
      - (2) Familial erythroblastic anemia (Cooley's anemia)
    - *e.* Sickle-cell anemia
  - 3. Hemoglobinuria
    - *a.* Paroxysmal types of hemoglobinuria
      - (1) Nocturnal hemoglobinuria
      - (2) March hemoglobinuria
      - (3) Paroxysmal (cold) hemoglobinuria
      - (4) Allergic hemoglobinuria
      - (5) Paralytic hemoglobinuria (myoglobinuria)
    - *b.* Symptomatic hemoglobinuria
      - (1) In bacterial and parasitic infection
      - (2) From incompatible blood transfusion
      - (3) From drugs and chemicals
      - (4) From animal poisons
      - (5) From vegetable poisons

- II. Deficiency anemias (hypoplasia of the bone marrow)
  - A. Iron-deficiency anemias (usually hypochromic and microcytic)
    - 1. Nutritional anemias
      - *a.* Hypochromic nutritional anemia of adults
      - *b.* Hypochromic nutritional anemia of children
      - *c.* Hypochromic nutritional anemia of pregnancy

- 2. Anemias due to faulty absorption of iron
    - *a.* Anemia of celiac diseases (chronic diarrhea, ulcerative colitis, and so on)
    - *b.* Simple achlorhydric anemia (idiopathic, microcytic, and hypochromic anemia)
    - *c.* Anemia associated with intestinal parasitism (hookworm, and so on)
- 3. Anemia due to defective storage of iron in the liver
    - *a.* Anemia of prematurity
    - *b.* Anemia of twins
    - *c.* Anemia of offspring of anemic mothers

B. Deficiency of antianemic factor (usually characterized by macrocytic hyperchromic anemia)

- 1. Deficiency of intrinsic gastric factor
    - *a.* Pernicious anemia
    - *b.* Certain types of anemia of pregnancy
    - *c.* Macrocytic anemias resulting from stomach and intestinal pathology
    - *d.* Macrocytic anemia of hypothyroidism
- 2. Deficiency of extrinsic factor
    - *a.* Tropical macrocytic anemia of pregnancy
    - *b.* Certain types of macrocytic anemia of pregnancy
    - *c.* Macrocytic anemia of sprue and pellagra
- 3. Deficiency due to faulty absorption of hemopoietic factors
    - *a.* Macrocytic anemia of pregnancy
    - *b.* Macrocytic anemia of intestinal parasites (such as Diphyllobothrium latum)
    - *c.* Macrocytic anemia associated with sprue
    - *d.* Macrocytic anemia of pellagra
    - *e.* Macrocytic anemia of celiac disease (idiopathic steatorrhea)
- 4. Anemia due to deficient storage of X factor in liver
    - *a.* Macrocytic anemias of liver damage (cirrhosis, pregnancy with toxemia, and phosphorous and chloroform poisoning)

III. Failure to use the antianemic principle

- A. Achrestic anemia

IV. Anemias of bone-marrow damage and dysfunction

- A. Due to toxic action of benzene, arsphenamine, gold compounds, infections, nitrogen retention, and so on
- B. Due to injury by x-ray and radium
- C. Due to disturbance of function in malignancies and osteosclerosis

D. Idiopathic dysfunction of the bone marrow
  1. Aplastic anemia
  2. von Jaksch's anemia (anemia pseudo-leukemia infantum)
  3. Splenic anemia (Banti's disease)
  4. Agnogenic myeloid metaplasia

Kolmer[2] has grouped the anemias as normocytic, macrocytic, simple microcytic, and hypochromic microcytic. The various types of anemias that he gives under these headings are as follows:

I. Normocytic:
  - Posthemorrhagic anemia
  - Simple chronic anemia
  - Scurvy, hemophilia, or purpura
  - Anemia from chemical, parasitic or bacterial poisons
  - Anemia from incompatible transfusions
  - Paroxysmal hemoglobinuria and hemoglobinemia
  - Lederer's acute hemolytic anemia
  - Anemia with congenital hemolytic jaundice
  - Anemia with cirrhosis of the liver
  - Sickle-cell anemia
  - Aplastic anemia (primary and secondary)
  - Anemia from acute, subacute, and chronic infections
  - Anemia in acute and chronic nephritis
  - Familial splenic anemia (Gaucher's disease)
  - Myelophthisic anemias
  - Purpura haemorrhagica
  - Anemia of pregnancy
  - Anemias associated with splenomegaly
  - Acute and chronic leukemia

II. Macrocytic:
  - Pernicious anemia
  - Anemia from sprue, idiopathic steatorrhea, celiac disease, or chronic diarrhea
  - Anemia from pellagra
  - Anemia from dietary deficiencies
  - Anemia of pregnancy
  - Anemia of diphyllobothrium-latum infection
  - Anemia of hypothyroidism
  - Anemia in chronic and extensive hepatic disease
  - Achrestic anemia
  - Some cases of aplastic anemia
  - Sickle-cell anemia
  - Erythroblastosis foetalis
  - Acute hemolytic anemia
  - Chronic hemolytic anemia
  - Multiple myeloma
  - Leukemia

III. Simple microcytic:
  - Anemia from subacute and chronic inflammatory and chronic noninflammatory diseases
  - Familial erythroblastic anemia (Cooley's disease)

IV. Hypochromic microcytic:
  - Anemia from iron deficiency in diet (especially in infants)
  - Anemia from achlorhydria
  - Anemia in gastrectomy (total or partial)
  - Anemia in sprue, celiac disease and chronic diarrhea
  - Anemia in multiple hereditary telangiectasia
  - Anemia of repeated pregnancies
  - Anemia of chlorosis
  - Chronic anemia of women
  - Hypochromic anemia of infants
  - Familial erythroblastic anemia (Cooley's disease)
  - Anemia from chronic hemorrhage
  - Hemolytic anemias

ANEMIA DUE TO BLOOD LOSS

EXTRAVASCULAR LOSS

Acute Hemorrhage:

In a moderate hemorrhage, the red-blood-cell count and volume of packed cells are higher than would be expected because of the release of erythrocytes, particularly from the spleen. Within a few minutes, there is an increase in the number of platelets. When the fluid volume is readjusted by administration of intravenous or oral fluids, the red-blood-cell count is decreased. In twenty-four to forty-eight hours, the bone marrow responds to the decrease in erythrocytes by producing more cells. This results in an increase in the number of reticulocytes in the peripheral blood. By this time, the leukocytes may reach as high as 25,000 per cubic millimeter, and the differential count shows an increase in nonsegmented neutrophils (metamyelocytes).

The blood picture following acute loss of blood is seen in blood donors. As mentioned above, there are temporary changes in the fluid and cellular elements, but these correct themselves in a short time. The donation of 500 cc. of blood no oftener than once every one or two months shows no permanent reduction of the blood elements in healthy persons over a period of one or two years.

Laboratory Examinations:

1. Decrease in the red-cell count and hemoglobin after fluid volume has been readjusted
2. Increase in the number of platelets immediately

3. Slight increase in number of reticulocytes after twenty-four to forty-eight hours
4. Increase in leukocytes to as many as 25,000 per cubic millimeter after twenty-four to forty-eight hours. They show an increase in nonsegmented polymorphonuclear leukocytes

Chronic Hemorrhage:

Bleeding from peptic ulcer, hemorrhoids, uterus (menorrhagia and metrorrhagia), or carcinoma, and in purpura or hemophilia results in hypochromic anemia. This type of anemia is characterized by a decrease in the number of erythrocytes with a greater decrease in hemoglobin because of the hypochromia. This brings about a low color index. The blood smear usually shows a microcytosis; and since the function of the bone marrow is depressed, the reticulocytes are decreased. In more extreme cases, there will be variation in size (anisocytosis) and shape (poikylocytosis).

INTRAVASCULAR DESTRUCTION AND HEMOLYSIS

Symptomatic Hemolytic Anemias:

Acute and Chronic Bacterial Infections: A massive infection of the bloodstream with a Clostridium or hemolytic streptococcus causes destruction of the erythrocytes. The organism or its toxins also depresses the bone-marrow function. Chronic infections, such as tuberculosis, syphilis, undulant fever, subacute bacterial endocarditis, and ulcerative colitis, also cause hypochromic anemia with a picture similar to that described under Chronic Hemorrhage (p. 330).

Parasitic Infections. OROYA FEVER: This is an acute hemolytic anemia called "Oroya fever," found in Peru. It is caused by Bartonella bacilliformis, which is transmitted to humans by the sandfly, Phlebotomus, and possibly by other arthropods. The mortality rate is high (30 to 98 per cent).

MALARIA: The Plasmodium invades the erythrocytes and destroys them by breaking them up mechanically. In chronic malaria, the enlarged spleen causes destruction of red cells at a faster rate than normal. As a result of both processes, hypochromic anemia develops.

BLACKWATER FEVER: Blackwater fever is a form of acute hemolytic anemia with hemoglobinuria which develops in connection with

Plasmodium-falciparum infection. The disorder has been attributed to desensitization to protein antigens of the malarial parasite produced by earlier infections.[3] It has also been found that the normal inhibitory effect of serum against lysis is reduced in blackwater fever.

Incompatible Blood Transfusion: With the administration of incompatible blood, the recipient develops restlessness, anxiety, flushing of the face, precordial pain, increase in pulse and respiratory rate, generalized tingling sensation, and pain in the back and thighs. Later, there may be nausea and vomiting, shock with a cold moist skin, coma, and a failing pulse. A chill followed by a temperature rise and possibly delirium may take place. Hemoglobinemia and hemoglobinuria with oliguria may develop. Leukopenia is followed by leukocytosis. The patient may progress slowly to a state of uremia, and the blood will show a high nonprotein nitrogen.

Caused by Drugs and Chemicals: Certain drugs and chemicals cause anemia by direct hemolytic action on the red cells. Among the drugs resulting in hemolytic anemia are sulfonamides (in certain susceptible persons), organic arsenic compounds, pyridine, phenylhydrazine, acetylphenylhydrazine, potassium chlorate, trinitrotoluene, dinitrobenzene, and acetanilid.

Among the chemicals which cause poisoning with an associated hemolytic anemia are lead (see p. 318), arseniuretted hydrogen, and trinitrotoluene. Arseniuretted-hydrogen poisoning, in addition to hemolytic anemia, results in a normal white count or a leukopenia and the formation of methemoglobin. Trinitrotoluene poisoning results in increased stippling, reticulocytosis, peripheral bloodstream normoblasts, and formation of methemoglobin.

From Animal Poisons: Certain types of snake venom cause severe hemolytic anemia with hemoglobinuria. According to Wilbur and Collier,[4] snake venom contains a lecithinase which converts lecithin o lysolecithin, a powerful hemolytic agent which may act by causing welling of the red cells.

From Vegetable Poisons: The ingestion of, or even inhalation rom, the blossoms of fava beans (Vicia fave) causes acute hemolytic nemia in sensitive persons. This disease, known as "favism," occurs n Southern Italy,[5] Sicily, Sardinia, and several cases have been reported in the United States.[6]

According to Lederer,[7] a hemolytic "spring anemia" in Baghdad is thought to be the result of sensitivity to the pollen of certain flowers.

Laboratory Examinations: The laboratory findings in most cases of symptomatic hemolytic anemia, which have lasted for some time, are as follows:

1. Erythrocytes: Reticulocytes and stippled cells are markedly increased because of an attempt by the bone marrow to compensate for the destruction of red cells. There is marked reduction in the number of erythrocytes, hemoglobin, and volume of packed red cells. The anemia may be normocytic or macrocytic at first, but later it becomes microcytic and hypochromic with a low color index. There is likely to be variation in shape of erythrocytes, and blast forms will be present in large numbers if the bone marrow is functioning satisfactorily. Spherocytes are present in some cases. The fragility of erythrocytes is normal or only slightly increased.
2. Leukocytes: The white cells are usually increased in number, and myelocytes with an occasional myeloblast of the neutrophilic series may be seen. A normal white count or leukopenia is found in poisoning with arseniuretted hydrogen.
3. Bone Marrow: Examination of the sternal bone marrow reveals marked hyperplasia, particularly of young erythrocytes. The blast forms may be increased to three times the normal size.
4. Serum: Because of the increased rate of erythrocyte destruction and the inability of the liver to excrete it, hemoglobinuria and bilirubinemia are present. The icterus index and quantitative van den Bergh test are increased. The indirect van den Bergh test is positive, and the cholesterol is likely to be decreased.
5. Urine: Hemoglobin may be excreted in the urine (hemoglobinuria) in some cases. Albumin is present, and urobilinogen and urobilin are usually found in active cases.

Idiopathic Hemolytic Anemias:

Congenital Hemolytic Anemia: This is a congenital and often familial form of hemolytic anemia, characterized by increased fragility of red cells in hypotonic saline solutions.

LABORATORY EXAMINATIONS:

1. ERYTHROCYTES: The erythrocytes are unusually fragile when placed in solutions of hypotonic saline. Hemolysis begins at

0.72 to 0.50 per cent of sodium chloride and is complete at 0.40 or 0.39 per cent. In normal blood, hemolysis begins at 0.46 to 0.40 per cent and is complete at 0.36 to 0.30 per cent.

The reticulocytes are definitely increased. Nucleated red cells may be present when anemia is severe. The morphology of the erythrocytes is characteristically spherical rather than biconcave. The number of red cells is usually reduced, particularly during crises. The hemoglobin and cell volume are also reduced.

2. Leukocytes: Changes in the leukocytes are not diagnostic, but they are increased during a hemolytic crisis. Metamyelocytes are increased, and blast forms are present during such crises.
3. Bone Marrow: Examination of the bone marrow of the sternum reveals increased activity of the erythropoietic series, with increase in the number of blast forms. They are normoblasts or macroblasts and may number as high as 60 per cent of the total number of nucleated cells. (The normal number of nucleated red cells is 20 per cent).
4. Serum: The increased production of bilirubin from the hemoglobin of destroyed red cells results in an increase in the icteric index (16 to 30) and in a positive, indirect van den Bergh. Blood cholesterol is usually decreased.
5. Urine: The urine does not contain bile pigment or salts, but it does show an increase in urobilinogen and urobilin.
6. The Coomb's test[8] is positive in acquired hemolytic jaundice and negative in congenital hemolytic jaundice. The test is carried out by mixing 2 drops of a 2 per cent suspension of patient's cells with diluted antihuman rabbit serum. A macroscopic agglutination of the patient's cells within an hour is considered positive and indicative of acquired hemolytic jaundice. Failure to produce agglutination is indicative of congenital hemolytic jaundice.

Acquired Hemolytic Anemia: There is considerable difference of pinion as to the existence of an idiopathic, acquired, hemolytic nemia as a separate entity. It may develop spontaneously at various eriods of adult life without an hereditary factor, and it may be aused by one of the agents mentioned previously under Symptom-tic Hemolytic Anemias (p. 330). This type of hemolytic anemia is haracterized by sudden onset in an adult who has not had hemolytic nemia during childhood.

LABORATORY EXAMINATIONS:

1. The reticulocyte count is increased to a fairly high level, but the fragility of the red cells is normal.
2. Spherocytes are not seen, although the erythrocytes may be thicker than normal.

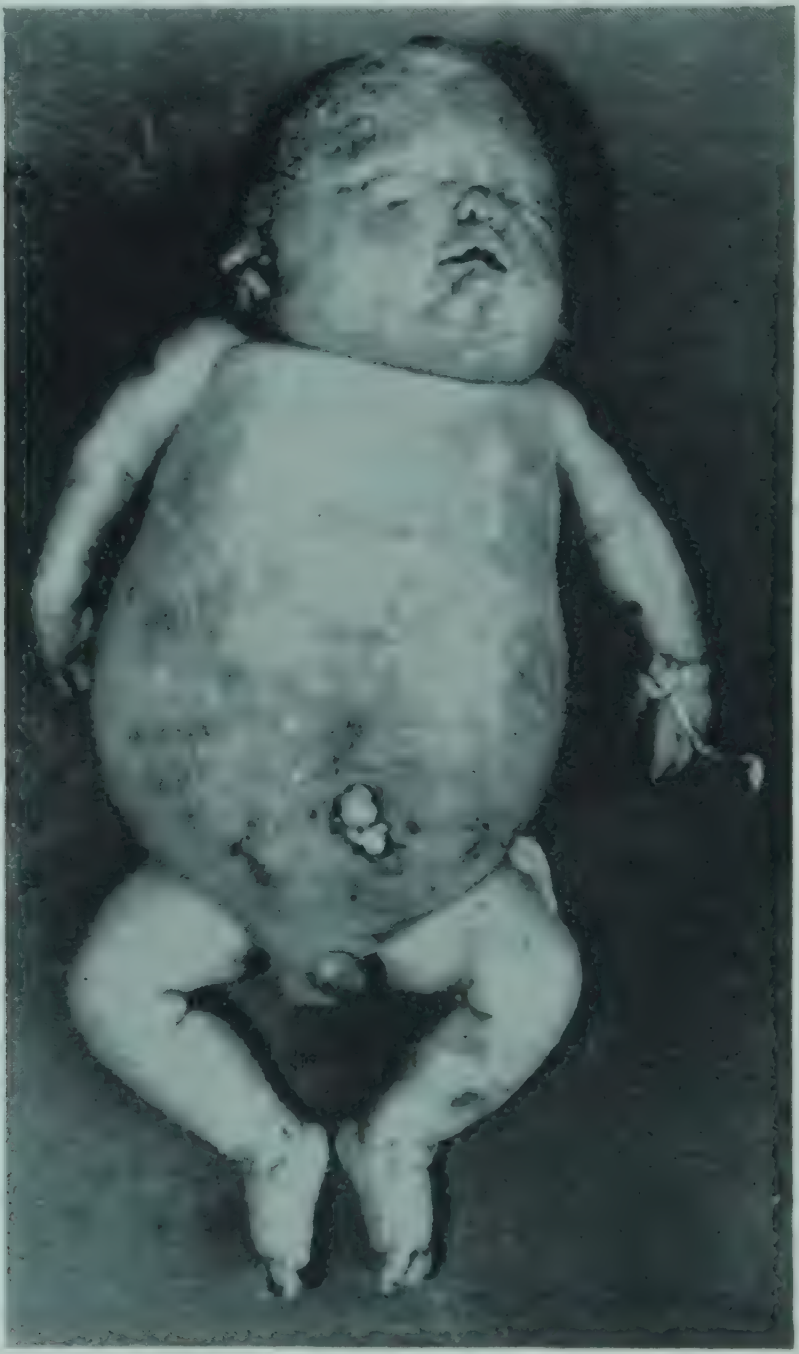

Fig. 1: Erythroblastosis foetalis of newborn infant.

3. This disease is further characterized by erythroblast showers, and showers of myelocytes and myeloblasts. In fact, the smear is sometimes such that the diagnosis of leukemia is entertained.
4. Urobilinogen and urobilin are increased in the urine.

Acute Hemolytic Anemia of Lederer: This disease was first described by Lederer in 1925. It is a rare type of hemolytic anemia, which is frequently accompanied by an infectious process and a fever.

The symptoms consist of vomiting, nausea, headache, coma, and muscle and abdominal pains. It responds readily to transfusions.

LABORATORY EXAMINATIONS: The laboratory findings are summarized as follows:

1. Marked increase in reticulocytes, to as high as 50 per cent, and also showers of erythroblasts.
2. The anemia may be extreme with a red-cell count of only 1,000,000.

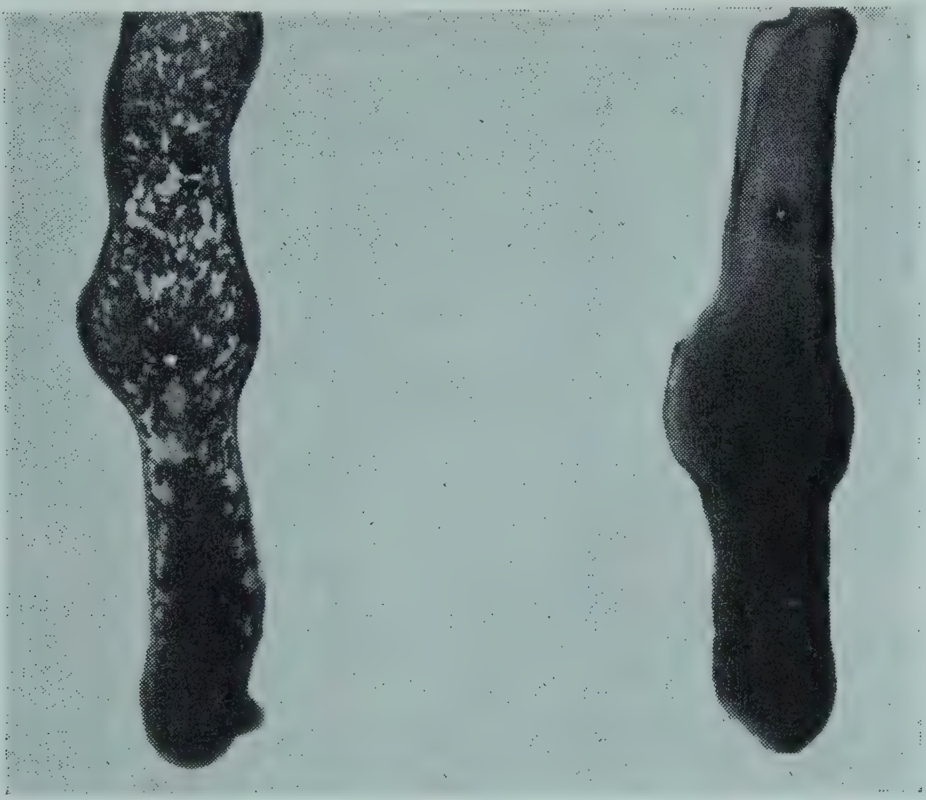

Fig. 2: Slide technic for determining Rh factor. *Left:* Rh positive. *Right:* Rh negative.

3. The white cells are increased, sometimes up to 50,000, with metamyelocytes, myelocytes, and an occasional myeloblast in the smear.
4. With the excessive hemolysis, there is bilirubinemia, increased icteric index, and positive, indirect van den Bergh.
5. Urobilinogen and urobilin are present in the urine, but bile pigments are absent. Albumin and casts may or may not be present.

Erythroblastic Anemia. ERYTHROBLASTOSIS FOETALIS: This is also known as "icterus gravis," "congenital anemia of the newborn," "hydrops fetalis," and "congenital hemolytic disease." Clinically, it is characterized by jaundice of the hemolytic type, edema, sometimes ascites, and large liver and spleen. There may be petechial hemorrhages and spastic paralysis.

Recent developments appear to take this disease out of the category of "idiopathic." It was stated in Chapter 3 (see p. 94) that congenital hemolytic disease is caused by Rh antibodies in the mother's blood. An Rh-negative woman married to an Rh-positive man can expect all of her children to be Rh-positive if her husband is homozygous, and half to be Rh-positive if he is heterozygous. Apparently, during pregnancy with the above Rh combination of parents, the blood of an Rh-positive fetus escapes into the maternal circulation. Antibodies may be produced by the mother against these cells. If any maternal blood passes into the fetal circulation, these antibodies may act upon the erythrocytes of the fetus and destroy them. This is believed to be the underlying process in the causation of erythroblastosis foetalis. The first child is rarely affected; but with increasing concentration of antibodies, subsequent children may develop the disease.

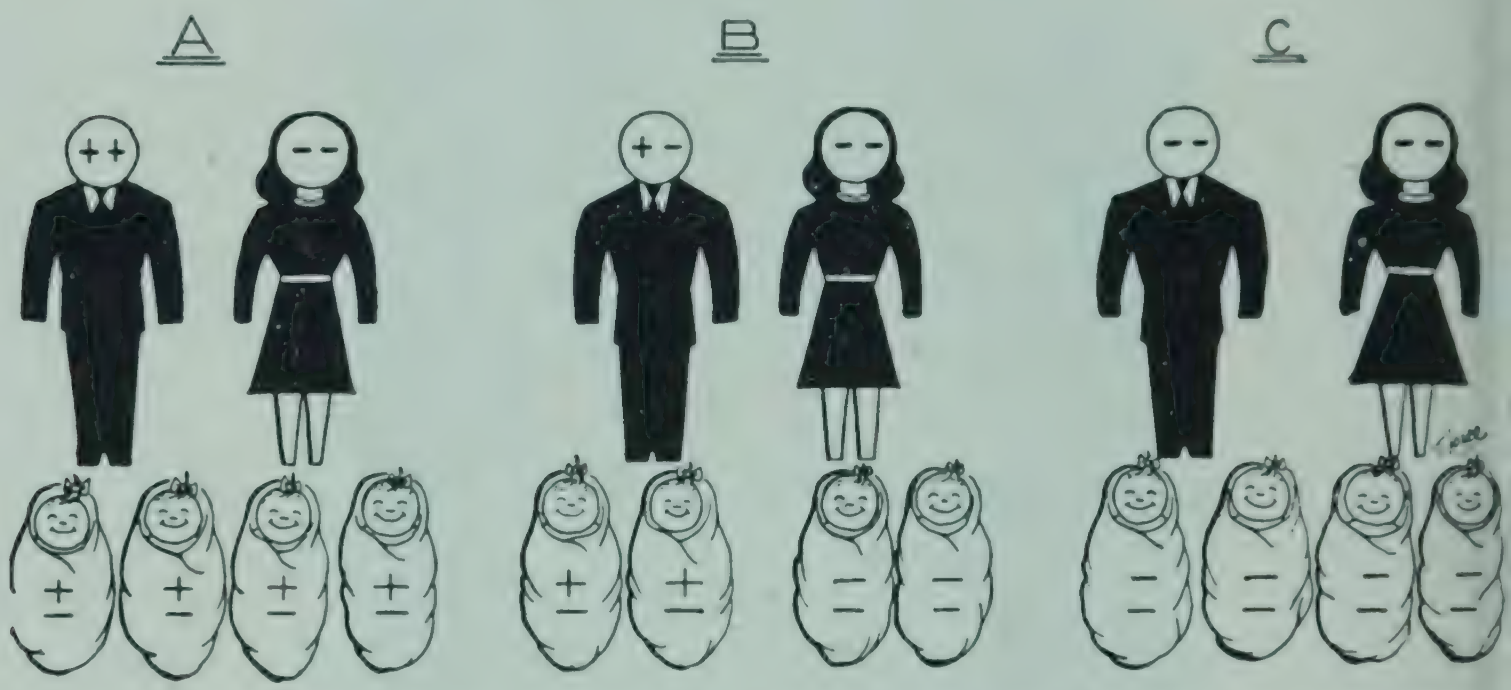

Fig. 3: Diagrams to illustrate the heredity of the Rh blood types. The mother is Rh-negative. In *A*, the father has a pair of Rh-positive genes and is accordingly homozygous. Every one of the children will carry one positive and one negative gene. Since the positive gene is dominant, all such offspring (even though hybrid or heterozygous) will be Rh-positive. In *B*, the father carries one positive and one negative gene and is accordingly heterozygous. Half of the children will be Rh-positive; the other half, Rh-negative. In C, both parents are Rh-negative and all children must be also Rh-negative. (Courtesy Samson Laboratories, Philadelphia.)

When a child is born with the above Rh combination of parents it is advisable to determine the presence or absence of Rh antibodies When Rh antibodies are demonstrated, it is assumed that the infant will sooner or later develop erythroblastosis (if it is not already present), and transfusion replacement therapy is instituted immediately.

Erythroblastic anemia is occasionally encountered in offspring of parents with major blood types; for example, a type A father and a type O mother.

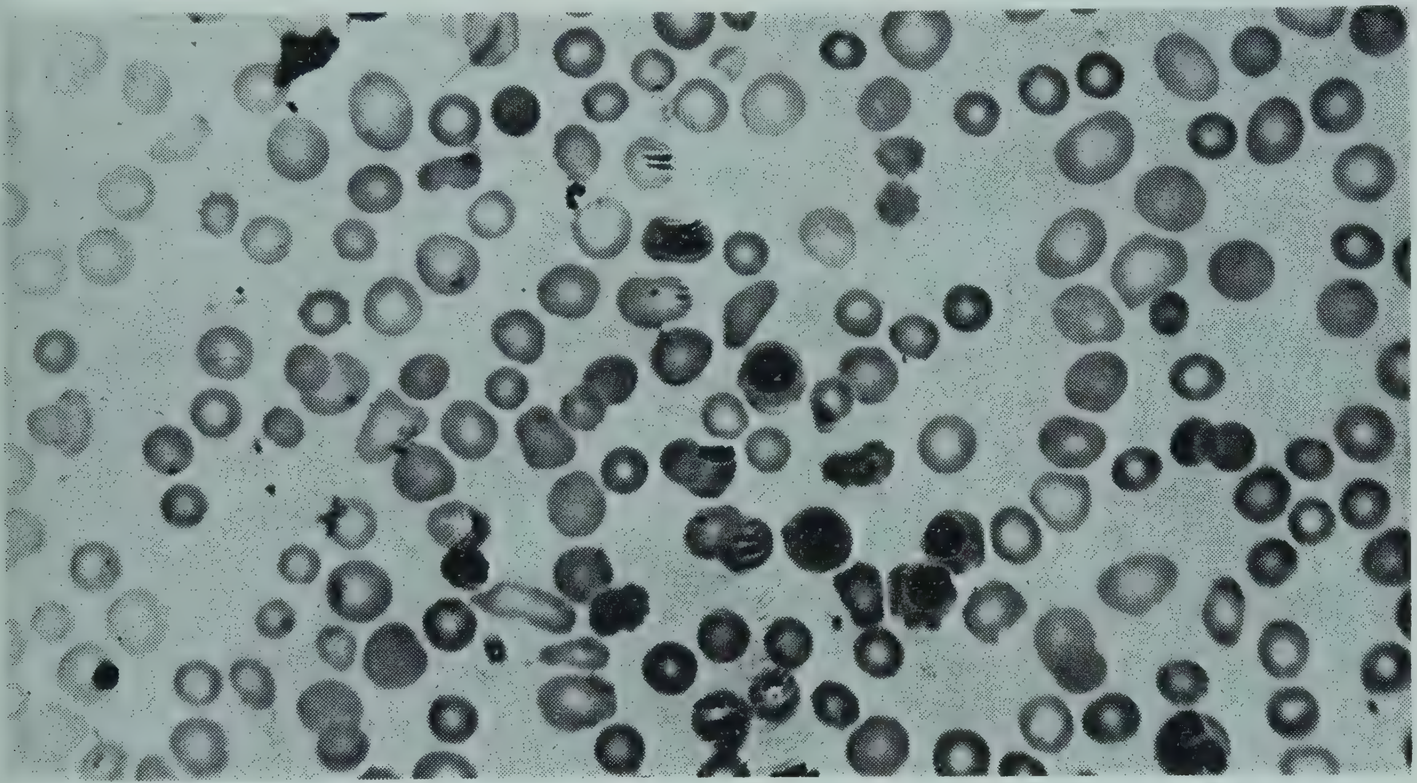

Fig. 4: Erythroblasts in a case of erythroblastosis foetalis.

Laboratory Examinations:

1. The outstanding characteristic in the blood is the presence of increased numbers of erythroblasts.
2. The red cells are markedly decreased. They show polychromatophilia, stippling, and an increase in reticulocytes.
3. Leukocytes are increased, and myelocytes and myeloblasts are usually present.
4. The icterus index is increased, and the van den Bergh test is positive, usually of the biphasic type. In addition to the presence of prehepatic bilirubin, there is apparently some posthepatic bilirubin owing to the liver-cell damage and partial obstruction of biliary passages.
5. If the baby appears to be Rh-negative on direct typing, a Coombs antiglobulin test should be performed. (A positive Coombs test indicates that the baby is Rh-positive, and that the direct typing test is falsely negative.)

FAMILIAL ERYTHROBLASTIC ANEMIA: This is also known as "Cooley's anemia," "Mediterranean anemia," or "target-cell anemia." It is first noted at the end of the first year and is characterized by pallor, excessive fatigue, anorexia, vomiting, hepatomegaly, splenomegaly, and sometimes fever. A Mongoloid facies is generally present. The disease goes on to a fatal termination.

Laboratory Examinations:

1. The important laboratory findings are in the blood smear, which contains nucleated red cells, "target cells," and an increase in reticulocytes.
2. The red cells are also decreased both in number and in volume of packed cells.
3. They are of the microcytic and hypochromic type. There is considerable polychromatophilia and variation in size and shape.
4. Leukocytosis is present, and myelocytes and myeloblasts may be seen.
5. Bilirubin is increased because of excessive hemolysis, and the icterus index is consequently moderately increased. The indirect van den Bergh test is positive.
6. Urobilinogen and urobilin are usually positive in the urine.

Sickle-Cell Anemia: This is a chronic, hemolytic type of anemia peculiar to the negro race and those with mixed blood. It is characterized by weakness, easy fatigue, nausea, vomiting, abdominal pain, and joint pains simulating arthritis. Leg ulcers are frequently found near the ankles.

LABORATORY EXAMINATIONS:

1. The diagnosis can be made by examination of the blood, since the outstanding feature is sickling of the red cells. In some cases, this characteristic morphology may be seen in the stained smear or in the counting chamber, but usually special preparations must be made. The usual method is to place a drop of blood on a slide, cover with a coverslip, ring with vaseline, and examine for sickling after twenty-four hours. The method of Hansen-Pruss[9] brings out the sickling much more quickly. A moist smear is prepared with brilliant cresyl blue as for a reticulocyte count; and the sickling, when present, is demonstrable in a short time. Collection of blood under oil in a test tube and fixation with formaldehyde according to Beck and

Hertz[10] reveals sickling in about twice as many persons as the other methods.

2. Additional findings in the blood are decrease in erythrocytes, hemoglobin, and volume of packed cells.
3. Although it may be macrocytic in severe cases, hypochromia with "target cells" is more common.
4. Nucleated red cells, polychromatophilia, stippling, and Howell-Jolly bodies are also seen. Sickle cells do not form rouleaux.

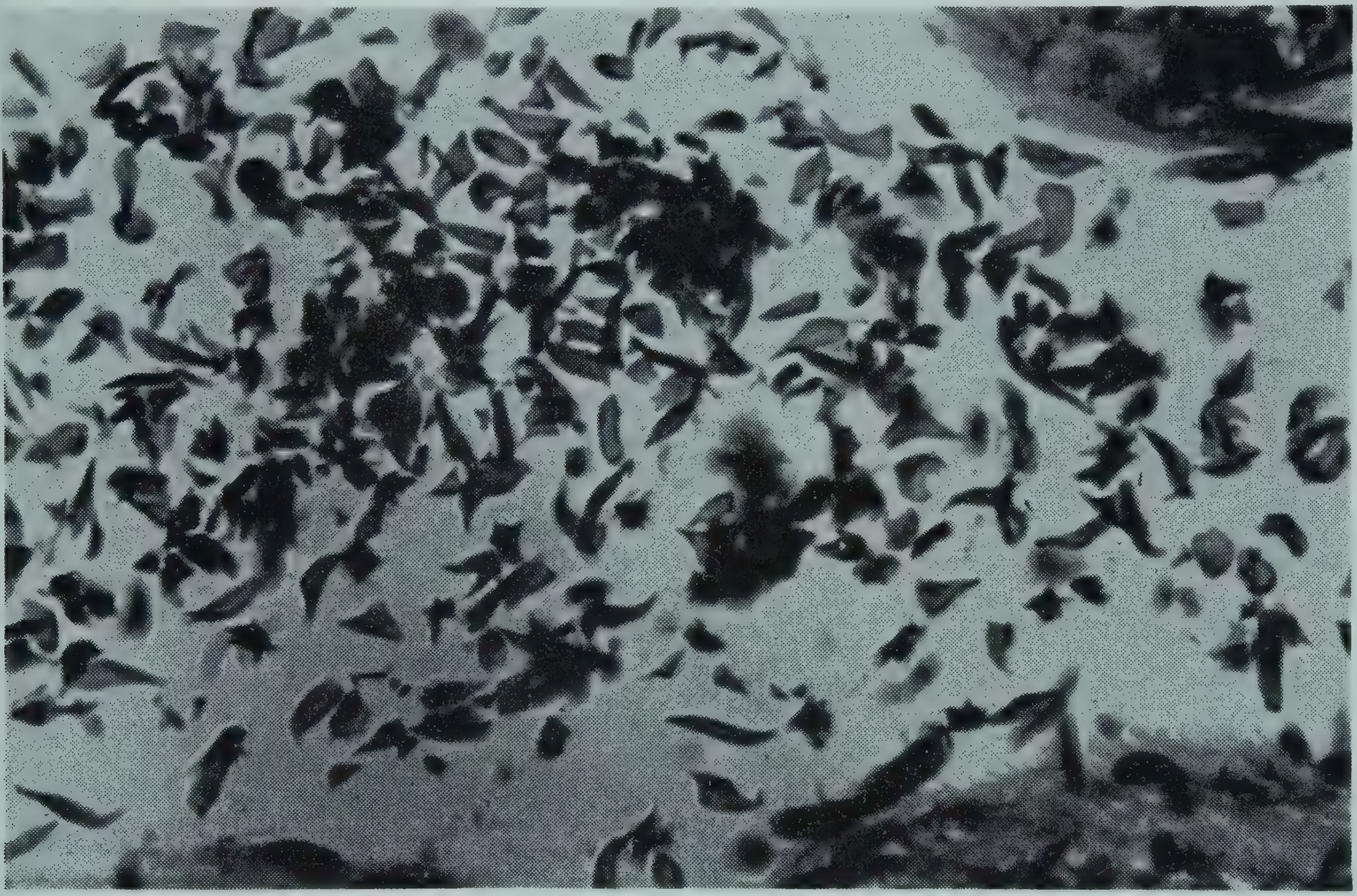

Fig. 5: Sickle cells.

5. Some cases show a leukocytosis with myelocytes and myeloblasts, and they may be confused with myelogenous leukemia.
6. Eosinophilia is present in some cases, and increase in monocytes is also seen.
7. The examination of sternal marrow (see also p. 7) reveals an increase in erythroblasts up to 70 per cent, some of which, according to Sharp and Schleicher,[11] are atypical in shape.
8. There is also excessive destruction of red cells, and consequently there is a bilirubinemia with increased icterus index and positive, indirect van den Bergh.
9. There is increased urobilinogen and urobilin in the urine, which usually has a specific gravity fixed at 1.010 or lower, and there may be albumin and casts.

Hemoglobinuria:

Paroxysmal Types. NOCTURNAL HEMOGLOBINURIA: This is a syndrome of a chronic hemolytic anemia, and it is characterized by hemolysis and hemoglobinuria during sleep.

Laboratory Examinations:

1. The red count may be as low as 1,000,000 with corresponding hemoglobin and packed red-cell levels.
2. Reticulocytes are increased, and occasionally erythroblasts are seen.
3. White cells are likely to be on the low side of normal and to have a normal differential.
4. Bilirubin is increased, and the indirect van den Bergh is positive.
5. The urine is characterized by the presence of hemoglobin after awaking from sleep. The cause and mechanism are not known.

MARCH HEMOGLOBINURIA: This is a type of hemoglobinuria that follows physical effort. It has been noted in soldiers after long marches. It usually occurs about two hours after cessation of exercise. The syndrome appears to be independent of diseases, chemicals, food, and cold. In addition to the hemoglobin, the urine may show red cells. The destruction of red cells is not sufficient to cause anemia. The susceptibility disappears after a few months, but it may recur.

PAROXYSMAL (COLD) HEMOGLOBINURIA: Paroxysmal hemoglobinuria manifests itself from a few minutes to eight hours after exposure to the cold. The attacks are characterized by cramps, headache, malaise, vomiting, or diarrhea, followed by chills and fever. The subject is weak and pale. Spleen and liver are frequently palpable. It is usually a manifestation of congenital syphilis, but has been seen in the acquired form of the disease.

Laboratory Examinations:

1. The serological test for syphilis is positive.
2. There is a hypochromic anemia with a red count as low as 2,000,000 per cubic millimeter.
3. The white-cell count falls during exposure to cold, and leukocytosis follows the chill. There is often definite shift to the left in the differential count.
4. Bilirubinemia is present, and the indirect van den Bergh is positive.
5. Hemoglobinuria is present during an attack. The urine contains both oxyhemoglobin and methemoglobin, which give it a brown

to black appearance. The urine sediment contains shadows of red cells and red-cell casts. Albumin is positive. These findings may be present only in the first or second specimen of urine passed after an attack.

6. Immersion of the hands or feet in ice water for ten to twenty minutes will produce an attack (Rosenbach test).
7. The hemolysins in the serum may be demonstrated as follows: Withdraw 10 cc. of blood from the patient; place half in a dry test tube and the other half in a tube containing ammonium and potassium oxalate. Some of the oxalated red cells are washed three times in warm, 0.85 per cent, sodium-chloride solution, and then 2 cc. of a 5 per cent suspension is made up. A normal control from a homologous blood group is prepared in the same way.

   Fresh guinea-pig serum is prepared in a 1:10 dilution for complement.

   Six Kahn tubes are placed in a rack. Place in each 0.2 cc. of complement. Place 0.1 cc. of physiological saline solution in tubes 1, 2, 3, and 4, and 0.06 cc. in tubes 5 and 6. Pipet 0.5 cc. of patient's serum in tubes 1 and 3. Pipet 0.2 cc. of patient's red cells in tubes 1, 4, and 5. Place 0.5 cc. of control serum in tubes 2 and 4. Next put 0.2 cc. of control red cells in tubes 2, 3, and 6. Immerse all tubes in ice water for ten to thirty minutes, and then in a 37° C. (98.6° F.) waterbath for thirty minutes.

   In a positive test, tubes 1 and 3 will show hemolysis, while the remaining tubes will be clear (no hemolysis).

ALLERGIC HEMOGLOBINURIA: This is caused by the ingestion or inhalation of the pollen of the bean plant Vicia fave. It appears to be an allergic response because the hemoglobinuria does not occur following the first exposure. It occurs in Sardinia, Sicily, and southern Italy where this plant grows.

Jaundice and hemoglobinuria develop within twenty-four hours and last three or four days. Splenomegaly may or may not be present.

Laboratory Examinations:

1. The urine may contain red cells in addition to the hemoglobin.
2. A marked anemia develops with a red count of 1,000,000 or less.
3. Bilirubinemia is present, and the inderect van den Bergh is positive.

PARALYTIC HEMOGLOBINURIA: This is a rare disease of unknown etiology, which is characterized by the passage of dark urine containing myoglobin. The patient has muscle weakness, cramps, and occasionally paralysis. Myoglobinuria is attributed to the sudden release of lactic acid from excessive amounts of glycogen, which formed in the muscles during rest. The lactic acid damages the myofibrillae and allows myoglobin to escape. There are both myoglobinemia and myoglobinuria. Since there is no hemolysis of red cells, there is no decrease in the red count and no increase in reticulocytes.

Symptomatic Hemoglobinuria: See Symptomatic Hemolytic Anemias (pp. 330-332).

DEFICIENCY ANEMIAS

IRON-DEFICIENCY ANEMIAS
(USUALLY HYPOCHROMIC AND MICROCYTIC)

Nutritional Anemias:

Hypochromic Nutritional Anemia of Adults: An inadequate diet or a diet lacking in iron can occasionally cause a hypochromic anemia. Various conditions, such as gastrointestinal upsets, allergy to certain foods, and poor teeth, may bring about anemia in spite of the adequacy of the diet.

Hypochromic anemia is likely to be more common in those regions where the soil and crops are low in iron content.

Chlorosis: Chlorosis is a hypochromic microcytic type of anemia of young girls, which is not found as commonly now, probably because of better nutrition and working conditions. It is presumably due to a deficiency of iron and possibly other factors found in a well-rounded diet.

There is a moderate reduction in red cells with a greater reduction in hemoglobin, giving a color index of less than one. The erythrocytes are hypochromic and microcytic, and the volume of packed red cells is therefore reduced. Polychromatophilia and anisocytosis are common, but poikylocytosis does not occur. There is a normal amount of hydrochloric acid in the gastric contents.

Hypochromic Nutritional Anemia of Children: Hypochromic anemia is fairly frequent in children between six months and three years of age, especially in premature infants and in children of poor families. Older children suffer from hypochromic anemia when the diet is defective or if the eating habits are abnormal.

Hypochromic Nutritional Anemia of Pregnancy: The hypochromic type of anemia is more common in pregnancy than the macrocytic type. The causes of this anemia may be attributed to the following: (1) The patient may be anemic before conception, and the demands of the fetus emphasizes this condition; (2) physiological disturbances, such as nausea and vomiting, prevent normal intake of food and thereby contribute to the anemic state; and (3) toxemia with resulting liver and kidney damage results in improper metabolism and storage of hematopoietic agents.

Anemia Due to Faulty Absorption of Iron:

Anemia of Celiac Diseases: In disorders of the alimentary tract which prevent the proper absorption of iron, hypochromic anemia may develop. Such conditions are chronic diarrhea from any cause and ulcerative colitis.

Simple Achlorhydric Anemia: This is also known as "chronic hypochromic anemia," "essential hypochromic anemia," and "late chlorosis." Women are much more susceptible than men. The onset is insidious and prolonged. The patient complains of fatigue, weakness, headache, and disturbance of sleep. The appetite is frequently capricious, and there is frequently a craving for unusual food. Following meals, there may be flatulence, epigastric distress, and even heartburn. Constipation is fairly frequent. Difficulty in swallowing, accompanied by pain, is sometimes an important and vexing symptom and may interfere with nutrition. Sore mouth is present in some cases, but is not as severe as in pernicious anemia.

Other symptoms are palpitation, edema of the ankles or face, menorrhagia, dysmenorrhea, signs of hypothyroidism, neuralgic pains, numbness, and tingling.

In the physical examination, pallor is the outstanding characteristic. The skin, mucous membranes, and nailbeds are pale. There is some atrophy of the papillae of the tongue in about one third of the cases. The heart is slightly enlarged, and functional systolic murmurs are frequently heard. The liver and spleen are sometimes palpable. A striking feature in about one third of the cases is flattening of the fingernails and the formation of a longitudinal ridge. Brittleness and soreness are frequently associated with this type of nail. Sometimes they even become concave.

Anemia Associated with Intestinal Parasitism: Hookworm is the most widespread cause of hypochromic anemia. The amount of blood that the hookworm takes out of the system plays a very small part in the anemia. One theory states that hookworms elaborate a substance that is toxic for the bone marrow. According to another theory, the production of large numbers of eosinophils in the bone marrow leaves little space for the formation of red cells.

Sometimes, there is normal, or near normal, red-cell count, and the cells contain very little hemoglobin. This suggests that there is some interference with the manufacture of hemoglobin. In any event, when the parasite is eradicated, the anemia corrects itself in a relatively short time.

Anemia Due to Defective Storage of Iron in Liver:

Anemia of Prematurity: Premature infants appear to have an inadequate hemopoietic system. Defective storage of iron in the liver is probably not the entire cause. Lack of ability to utilize iron or to synthesize hemoglobin has been suggested by Abt and Nagel[12] as the cause of anemia in premature infants. Following the normal hemolysis which occurs after birth, the premature infants do not reach as high a level of red cells as normal infants.

Anemia of Twins: Twins with their lower-than-normal, individual birth weights fall into the same category as premature infants. Again the hemopoietic system appears to be inadequate to respond properly after the initial physiological hemolysis. The defect in the hemopoietic system is probably the same both in premature infants and in twins.

Anemia of Offspring of Anemic Mothers: According to Strauss,[13] hypochromic anemia is more frequent in children of anemic mothers than in children of healthy mothers. It has been usually accepted that an anemic mother is unable to bring about adequate iron storage during pregnancy. However, Davidson and Fullerton[14] found that at the end of a year the hemoglobin was lower when the birth weight was less than normal. This suggests that the hemoglobin level follows the birth weight rather than the anemia status of the mother.

Laboratory Examinations in Hypochromic Microcytic Anemia:

1. The red-blood-cell count ranges from 3,000,000 to 5,000,000 per cubic millimeter.
2. The hemoglobin is reduced out of proportion to the red-cell count because of the hypochromia. In some cases, it is as low as 6 to 10 gm. per 100 cc.
3. Because of this, the color index is reduced (0.5 to 0.8).
4. The size of the red cells is reduced to 50 to 80 cubic microns. The diameter is reduced to 6.2 to 7 microns.
5. The volume of packed red cells is reduced out of proportion to the red-cell count. It may be as low as 10 cc. per 100 cc.
6. The fragility of the red cells may be normal or may show increased resistance.
7. The bone marrow is hyperplastic with an absolute increase in normoblasts, particularly small polychromatophilic normoblasts. The granulocytes are essentially normal.

DEFICIENCY OF ANTIANEMIC FACTOR

Deficiency of Intrinsic Factor:

Pernicious Anemia: Pernicious anemia, also known as "primary anemia" and "Addisonian anemia," is a disease with insidious onset and characterized by the development of macrocytic anemia. In addition, the patients have achylia gastrica, glossitis, neurological and gastrointestinal disturbances, and signs of increased blood destruction. The response to liver therapy is so characteristic that without this response the diagnosis is open to doubt.

Castle and his colleagues[15] explained the interrelationship of the various macrocytic anemias which respond to liver therapy by a hypothesis based upon certain fundamental experiments. Castle showed that beef, when given to pernicious-anemia patients, mixed with gastric juice from normal persons, produced an effect similar to that brought about by liver. When beef and gastric juice were fed separately, at least six hours apart, no response took place. The specific substance in the gastric juice of pernicious-anemia subjects is lacking, and so the antianemic principle cannot be formed when beef alone is eaten. The name "intrinsic factor" was given to the specific substance in the gastric juice and "extrinsic factor" to the agent in beef muscle.

LABORATORY EXAMINATIONS:

1. ERYTHROCYTES: The red-cell count is markedly decreased during a relapse. Counts of less than 1,000,000 were not infrequent before the advent of liver therapy. The stained smear is characterized by the presence of hyperchromic macrocytes, although hypochromia may be present if there is an iron deficiency. Normocytes, microcytes, and variations in shape are also seen in the untreated case. Stippling, polychromatophilia, Cabot-ring bodies, Howell-Jolly bodies, and megaloblasts indicate that these cells have left the bone marrow before reaching maturity.

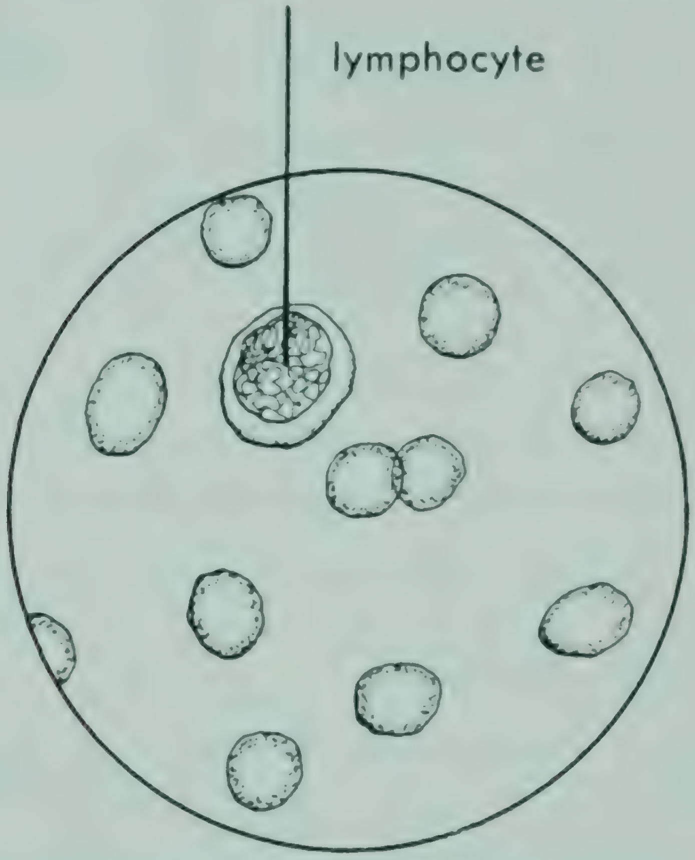

Fig. 6: Erythrocytes showing hypochromia.

The color index is greater than one, and the mean corpuscular volume is greater than 105. Reticulocytes may be slightly increased before liver therapy. After the institution of liver therapy, there is a progressive increase in reticulocytes to a peak and then a gradual recession, and the red count continues to increase toward normal. The reticulocyte response is so characteristic in pernicious anemia that it almost universally confirms the diagnosis. The reticulocyte response is in inverse ratio to the original red count. With an original red count of 1,000,000, the reticulocytes may reach 30 per cent or more: but with a red-cell count of 3,000,000, the reticulocyte response is not over 10 or 12 per cent at the peak.

The fragility of the erythrocytes is not significantly changed, but resistance to saponin hemolysis is decreased.[16]

2. Leukocytes: There is usually a leukopenia (2000 to 5000, with a reduction in granulocytes and a Schilling shift to the right. There may be a few myelocytes and occasionally a myeloblast. Eosinophils are increased during liver therapy.

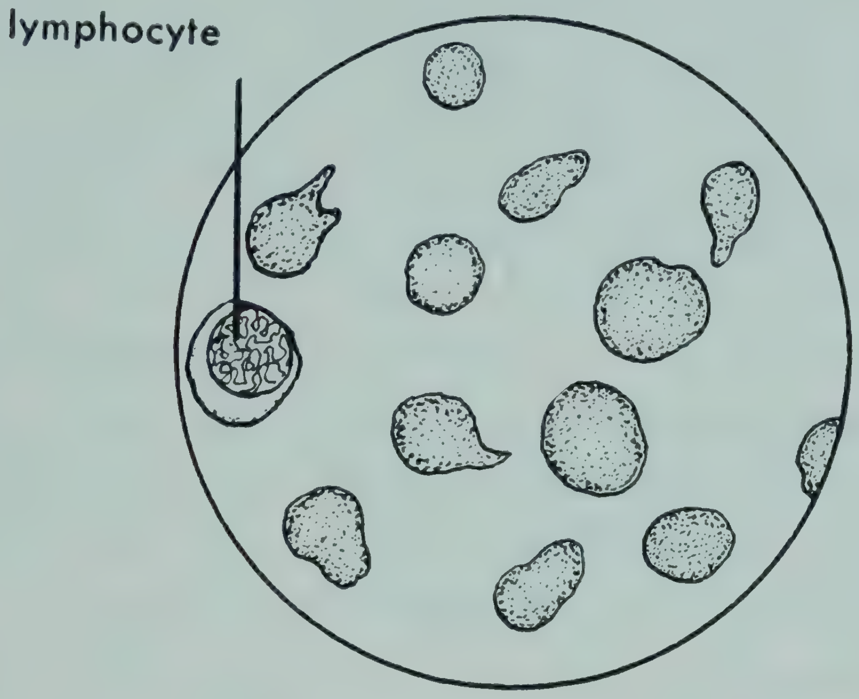

Fig. 7: Erythrocytes in pernicious anemia.

3. Platelets: Platelets are decreased in number, paralleling somewhat the decrease in red cells. The drop in platelets may bring about bleeding elsewhere in the form of purpuric spots and hemorrhages. The bleeding time is prolonged and clot retraction is delayed when the platelets are low. Platelets increase to normal after adequate liver therapy.
4. Serum: The serum in relapse has a deeper yellow color, the icteric index is increased to as high as 20, and bilirubinemia is present, which gives a positive indirect van den Bergh test.
5. Bone Marrow: Examination of the sternal bone marrow (see also p. 6) reveals hyperplasia of the erythropoietic series with up to 50 per cent of nucleated red cells. Many of these cells are megaloblasts. There is also active leukopoiesis, but the number of megakaryocytes is decreased.
6. Gastric Analysis: Achlorhydria after histamine stimulation should be present before the diagnosis of pernicious anemia can really be established. Absence of hydrochloric acid following an alcohol meal is not a sufficient criterion of achlorhydria. Histamine stimulation should be tried before the diagnosis is made.

7. URINE: During relapses the urine shows an increase in urobilinogen and urobilin. In addition, according to Dobriner and Rhoads,[17] porphyrin-I excretion is increased during a relapse and returns to normal after liver therapy. Albumin and casts are present, and the specific gravity is low and fixed.

Certain Types of Anemia of Pregnancy: Pernicious anemia of pregnancy is a rare disorder outside of the tropics. The etiology is obscure at present, although Strauss and Castle[18] offer some evidence to show that there is a temporary lack of the intrinsic factor. In the poorer classes, it is suggested that there is an inadequate amount of extrinsic factor in the diet. Other possibilities are a failure of interaction between the extrinsic and intrinsic factors because of vomiting of pregnancy, failure of absorption of the antianemic principle, or failure on the part of the liver to store and distribute the principle.

These patients may have most of the symptoms seen in pernicious anemia, such as sore tongue, pallor, fatigue, nausea, vomiting, diarrhea, and numbness and tingling of the extremities. Free hydrochloric acid is frequently present in the gastric contents.

LABORATORY EXAMINATIONS:

1. There is a decrease in the number of red cells with a high color index.
2. The mean corpuscular volume is increased because of the presence of macrocytes.
3. There is variation in size and shape, which is more marked with an extremely low red count.
4. Leukocytes and platelets are normal.
5. The gastric contents may show hypochlorhydria or achlorhydria.
6. There is a reticulocyte response after the administration of liver, and the red count gradually returns to normal. After delivery, the patient does not require liver therapy unless she has pernicious anemia.

Macrocytic Anemia Resulting from Stomach and Intestinal Pathology: It seems reasonable to assume that resection of the stomach or any pathological process which interferes with the secretion of the intrinsic factor would result in a macrocytic anemia. This is seen in gastrectomy, carcinoma of the stomach, and in a few cases of gastroenterostomy. In addition, Barker and Hummel[19] reported macrocytic anemia associated with a series of intestinal strictures and anastomoses. In such cases, the most likely explanation is inadequate absorption of the antianemic principle. Macrocytic anemia has also developed in cicatrizing enteritis, chronic dysentery, and following resection of intestine.

The blood picture is similar to that seen in primary pernicious anemia.

Macrocytic Anemia of Hypothyroidism: Anemia in cases of hypothyroidism may be macrocytic or normocytic. Achlorhydria occurs in about half of the cases of myxedema,[20] and anemia was more frequent in these patients. It is thought that the defective gastric secretion in cases of myxedema results in an insufficient amount of intrinsic factor and the development of macrocytic anemia.

Deficiency of Extrinsic Factor:

Tropical Macrocytic Anemia of Pregnancy: Wills[21] has reported on a form of pernicious anemia found chiefly among pregnant women of the tropics and subtropics. Later, the same type of anemia was reported by Napier[22] in nonpregnant women and in males usually young or of middle age. The cause of this type of anemia is apparently a dietary deficiency in which the extrinsic factor is lacking. In many cases, there is also an iron deficiency which confuses the picture. In uncomplicated cases, the blood picture simulates that of pernicious anemia.

Certain Types of Macrocytic Anemia of Pregnancy: As mentioned on page 348, one theory as to the cause of macrocytic anemia of pregnancy is a diet deficient in the extrinsic factor. This seems reasonable among those in the poorer classes, but some cases have been found in the upper economic level in patients with adequate food intake. Not all cases of this type of anemia can be explained on dietary deficiency alone.

See page 348 for laboratory examinations.

Macrocytic Anemia of Sprue and Pellagra: Sprue is a disease of the tropics and subtropics, which simulates pernicious anemia except for neurological signs. The cause appears to be a lack of the extrinsic factor in the food. It is a chronic wasting disease which is characterized by glossitis, diarrhea, and stools which are light-colored, bulky, and frothy.

The blood picture simulates that of pernicious anemia, that is a macrocytic hyperchromic anemia. There may be hypochromia in those persons who have a deficient iron intake. The best treatment is liver injection.

Pellagra is associated with alcoholism and an inadequate diet. Goldberger and Wheeler[23] believe it is due chiefly to a deficiency of vitamin B. Sydenstricker and associates[24] believe that there is also a gastric deficiency, since they produced remissions by administering gastric juice.

The blood picture is either macrocytic and hyperchromic, or normocytic and hypochromic. The red count is reduced, and the color index and mean corpuscular volume are usually increased. Achlorhydria may be seen in some patients, but it returns to normal after treatment.

Deficiency Due to Faulty Absorption of Hemopoietic Factors:

Macrocytic Anemia of Pregnancy: One theory of the cause of macrocytic anemia of pregnancy is faulty absorption of the hemopoietic factor. This, of course, presupposes an adequate diet (extrinsic factor) and a normally functioning gastric mucosa (intrinsic factor).

See page 348 for laboratory examinations.

Macrocytic Anemia of Diphyllobothrium Latum: A few persons infested with the fish tapeworm develop a macrocytic anemia which simulates pernicious anemia clinically and pathologically. Since only one in five thousand infested people develop macrocytic anemia, Birkeland[25] feels that the fish tapeworm is the precipitating factor, producing a pernicious-anemialike picture in persons who are already susceptible to the disease.

Macrocytic Anemia of Celiac Disease and Idiopathic Steatorrhea: Idiopathic steatorrhea and celiac disease simulate sprue in persons who have never been in the tropics. For this reason, "nontropical sprue" is used as a synonym for " idiopathic steatorrhea." In addition to glossitis, diarrhea, and light bulky stools, those with idiopathic steatorrhea have tetany due to calcium deficiency, osteoporosis, and osteomalacia. This disease is found in adolescents and adults, while the same picture in infants is called "celiac disease."

In idiopathic steatorrhea and celiac disease, the anemia may be either macrocytic or hypochromic microcytic. In view of the nature of these diseases, the macrocytic type is probably due to faulty absorption of the hemopoietic factor.

Anemia Due to Deficient Storage of X Factor in Liver:

Macrocytic Anemias of Liver Damage: Chronic liver disease, particularly cirrhosis, may be associated with macrocytic anemia probably because of the inability of the liver to store and synthesize the antianemic principle. The incidence of macrocytic anemia associated with liver disease varies from as low as 18 per cent to almost 100 per cent.[26] The disease apparently must be of long duration and very extensive before this type of anemia develops.

The blood picture is similar to that of pernicious anemia, but it is not usually so severe.

FAILURE TO USE THE ANTIANEMIC PRINCIPLE

Achrestic Anemia:

Achrestic anemia, described by Wilkinson and Israels[27] in 1935 in England, is similar to pernicious anemia, yet differs from it in certain respects. The red count is decreased to from 2,000,000 to 3,000,000. The mean diameter of the red cells is above normal, and the color index is greater than one. Occasional megaloblasts are seen, and there is variation in size and shape. Reticulocytes are reduced and do not increase even after liver therapy.

The disease differs from pernicious anemia in that there is free hydrochloric acid in the gastric contents, the patient does not respond to liver therapy, and adequate amounts of antianemic principle are present in the liver. Wilkinson and Israels have suggested that the underlying cause of the disease is a failure of the bone marrow to remove the antipernicious-anemia principle from the tissues and to utilize it.

The disease is chronic, it is not characterized by remissions, and death is the final outcome.

ANEMIAS OF BONE-MARROW DAMAGE AND DYSFUNCTION

Due to Toxic Action of Chemicals and Infection:

Secondary Aplastic Anemia: Secondary aplastic anemia may result from exposure to certain chemicals, such as benzene, arsphenamine, and gold compounds, mustard gas, severe septic processes, bone-marrow tumors, and prolonged hemorrhages. Susceptibility of different persons to bone-marrow depression by chemicals, particularly benzene, varies considerably. In some cases, therefore, true aplastic anemia may not develop, although there may be transient depression of bone-marrow function.

When aplastic anemia does develop, the red cells are decreased, but they do not show any appreciable change in size and volume. The hemoglobin level corresponds to the level of red cells. There is little or no evidence of regeneration (erythroblasts and reticulocytes) unless the person is beginning to recover.

Due to Injury by X-Ray and Radium:

Overexposure to x-ray or radium may result in a severe or irreversible aplastic anemia. Sufficient x-ray will cause a decrease in the number of all types of cells in the peripheral blood and will damage the bone marrow and lymph glands. The lymphocytes appear to be most sensitive to irradiation, with neutrophils and red cells less sensitive in that order. Platelets are less sensitive than neutrophils. There is sometimes an increase or slight decrease following x-ray irradiation, but a marked decrease is rare.

In studying radium-dial workers, Martland[28] found a blood picture which simulated severe macrocytic anemia. This effect was the result of chronic internal irradiation. The peripheral blood showed a leukopenia with a relative lymphocytosis and megaloblasts in the blood smear. The bone marrow showed hyperplasia of the erythroblastic and leukocytic series with an increase in megakaryocytes.

Due to Disturbance of Function in Malignancy and Osteosclerosis:

Myelophthisic Anemia: Myelophthisic anemia is one of the anemias of bone-marrow damage, and it is caused by metastatic carcinoma, multiple myeloma, osteoporosis, malignant granuloma, and xanthomatosis of the bone marrow.

LABORATORY EXAMINATIONS:

1. Examination of the blood reveals a marked decrease in the red count with a normocytic anemia.
2. The color index and mean corpuscular volume are not unusual.

3. The stained smear contains a number of normoblasts, and the reticulocytes are increased. Polychromatophilia and stippling are present.
4. The white-cell count is variable from a leukopenia to a leukocytosis.
5. The smear shows a normal proportion of granulocytes with myelocytes and occasionally a myeloblast.
6. The platelet count is frequently reduced so that hemorrhages may occur.
7. A biopsy of the bone marrow may be of help in establishing the diagnosis by revealing metastatic or primary tumor.

Osteosclerotic Anemia: This disease is characterized by increased density of the bones due to connective-tissue proliferation and deposition of bone, causing a decrease in area of the bone marrow. The cause of this activity is not known. A severe anemia and leukopenia result because of the limited amount of remaining bone marrow. Fortunately, this disease is very rare, since no therapy has thus far proved successful.

Idiopathic Dysfunction of Bone Marrow:

Idiopathic Aplastic Anemia: This is a disease of unknown cause and is characterized by weakness, pallor, and a severe repression of all of the bone-marrow elements (see p. 7).

LABORATORY EXAMINATIONS:

1. Laboratory investigation is concerned with the blood, which shows a decrease of all cellular elements, leukocytes, erythrocytes, and platelets. The red-cell count may be 1,000,000 or less, the white cells may be 3000 or less, and the platelets may be less than 50,000. The hemoglobin parallels the red count so that the color index is usually one.
2. The stained smear shows only a few erythrocytes in each field, but they are of normal size and do not vary in shape.
3. There is a decrease or absence of granulocytes with a relative lymphocytosis.
4. Platelets are decreased, and they are larger than normal.
5. The decrease in platelets results in a prolonged bleeding time, delayed clot retraction, and a positive tourniquet test for capillary resistance. The clotting time is normal.

von Jaksch's Anemia: Familial erythroblastic anemia (Cooley's anemia) has been separated from von Jaksch's anemia and is described elsewhere (see p. 338). The disease as described by von Jaksch is not thought to be a separate entity by some authorities.

According to Parsons and Hawksley,[29] it is an anemia of late infancy, characterized by waxy pallor, edema of feet and ankles, petechial hemorrhages, splenomegaly, and moderate hepatomegaly. Bone changes are not present.

LABORATORY EXAMINATIONS:

1. The blood examination reveals a severe anemia, usually of the macrocytic hyperchromic type.
2. The stained smear shows numerous megaloblasts, reticulocytes, stippled cells, polychromatophilia, and variation in size and shape.
3. There is a leukocytosis with many myelocytes and metamyelocytes.
4. Bilirubinemia is present.
5. There is an increased amount of urobilinogen in the urine.

Splenic Anemia (Banti's Disease): There is still much controversy as to whether or not this disease is a separate entity. It is characterized by splenomegaly, liver atrophy, sometimes hematemesis, and leukopenia. Although it occurs frequently in childhood, it is not limited to any particular age group.

LABORATORY EXAMINATIONS:

1. The white count may be as low as 2000, and the platelets may be normal or decreased.
2. The blood picture is characterized by a hyperchromic macrocytic anemia with increase in color index and mean corpuscular volume.

Agnogenic Myeloid Metaplasia: This is an anemia that may well be considered under idiopathic dysfunction of the bone marrow, although some believe it is a result of a bone-marrow toxin. It is characterized by splenomegaly, anemia, hypoplastic bone marrow, and sometimes jaundice. As a result of the inability of the bone marrow to supply blood cells in adequate numbers, the spleen and liver attempt to take over the hematopoietic function. Since the cells produced by the spleen are needed by the body, splenectomy is contraindicated. The spleen and liver do not have the regulatory mechanism of the bone marrow; thus young cells not normally found in the peripheral blood are found in smears.

LABORATORY EXAMINATIONS:

1. Erythroblasts, myelocytes, and even myeloblasts are found out of proportion to the degree of anemia. Twenty or more normoblasts per 100 white cells are not unusual. Megaloblasts are sometimes seen.

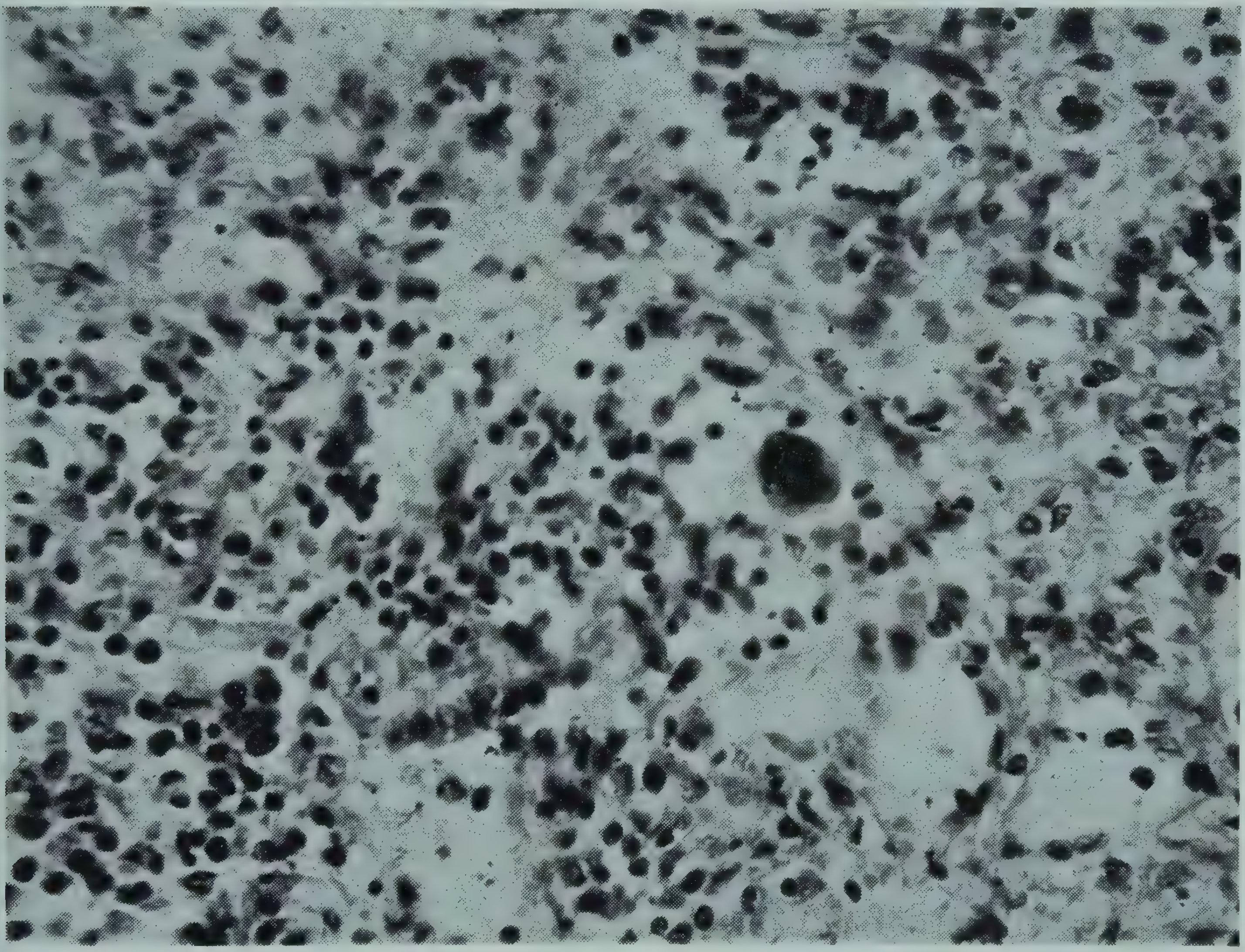

Fig. 8: Section of spleen from case of agnogenic myeloid metaplasia. Note megakaryocyte and nests of marrow cells.

2. There is a moderate normocytic anemia.
3. Reticulocytes are moderately increased, and polychromatophilia and stippling are present.
4. The white-cell count is variable, decreased, normal, or increased, and the granulocytes maintain their normal proportions.
5. The platelet count may be normal or reduced. I have confirmed the observations of others in finding giant platelets in such a case.

The following findings were observed by me in a case of agnogenic myeloid metaplasia in a thirty-four-year-old female who gave no history of exposure to a bone-marrow toxin:

Red-cell count 4,600,000 to 3,600,000 per cubic millimeter

White cell count: 16,900 to 23,650

Blood smear:	Eosinophils	3
	Myeloblasts	3
	Myelocytes	2
	Metamyelocytes	19
	Segmented polymorphonuclear leukocytes	68
	Lymphocytes	5
	Nucleated red cells	16 per 100 White Blood Cells
	Reticulocytes	7 to 9 per cent
	Platelets	many giant forms

Red cells show polychromatophilia, stippling, hypochromia, and slight variation in size and shape.

Splenectomy was done at the time (1936), although at present it is recognized that it is contraindicated. The spleen weighed 1100 gm. (2½ pounds). The microscopic section showed megakaryocytes and foci of nucleated red cells with young forms of the granulocytic series. The pathological diagnosis was "myeloid metaplasia."

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CHAPTER 19

The Blood and Hematopoietic Organs (Continued)

HEMORRHAGIC DISEASES

The causes of spontaneous bleeding may be divided into three categories: (1) failure of formation of the clot (deficiency of thromboplastin, prothrombin, or fibrinogen; (2) failure of clot to retract (deficiency of platelets); and (3) capillary fragility.

CLASSIFICATION

Under these categories, hemorrhagic diseases may be classified as follows:

- I. Deficiency of thromboplastin, prothrombin, or fibrinogen
  - A. Hemorrhagic disease of the newborn
  - B. Vitamin-K deficiency
  - C. Hereditary pseudohemophilia
  - D. Severe sepsis
  - E. Liver diseases (fatty degeneration; poisoning with chemicals, such as chloroform or phosphorus; and acute yellow atrophy)
- II. Deficiency of platelets
  - A. Idiopathic purpura hemorrhagica (Werlhof's disease)
  - B. Congenital thrombocytopenia

  - C. Symptomatic purpura (aplastic anemia, myelophthisic anemia [metastases of carcinoma or malignant granuloma to bone marrow], leukemia, certain infections, Gaucher's disease, Niemann-Pick disease, Hand-Schüller-Christian disease, splenomegaly of undetermined origin [Banti's disease], chemical agents [arsenic or benzene], physical agents [x-ray or radium], venoms from snake and insect bites, and following burns in some cases)

- III. Capillary fragility
  - A. Idiopathic (hereditary hemorrhagic telangiectasia)
  - B. Symptomatic
    1. Allergic (nonthrombopenic purpura [Henoch's purpura] and purpura rheumatica [Schönlein's purpura])
    2. Nutritional (vitamin-C deficiency and protein deficiency)
    3. Infections (focal or generalized acute infections)
    4. Toxic drugs, chemicals, and venenes
    5. Chronic cardiorenal disease
    6. Hemochromatosis
    7. Purpura senilis
    8. Purpura cachetica
    9. Purpura simplex
    10. Purpura fulminans
    11. Certain skin disorders

Hemophilia will be discussed separately because the exact defect in the clotting mechanism is not known at present.

DEFICIENCY OF THROMBOPLASTIN, PROTHROMBIN, OR FIBRINOGEN

Hemorrhagic Disease of the Newborn:

This disease is believed to be due to a deficiency of prothrombin, and this deficiency appears to be due to inadequate vitamin-K intake by the mother. Administration of vitamin K to mothers before delivery increases the prothrombin level of infants so that fewer infants develop the disease.

The disease is not inherited, it is self-limited, and it is characterized by spontaneous external and internal hemorrhages.

Laboratory Examinations:

1. The prothrombin level (see p. 32) is reduced as evidenced by a prolonged prothrombin time, ranging from 100 seconds to more than six minutes by the method of Quick. The prothrombin

time in the first 24 hours of life by the Quick technic is longer than that of adults. According to Kato and Poucher,[1] it is 43 seconds in normal, full-term infants and 46 seconds in premature infants. By the tenth day of life, the average was 25 seconds, according to these authors.

2. The bleeding (see p. 31) and coagulation times (see p. 29) are prolonged in most cases, although these findings are not absolutely necessary in making a diagnosis.
3. Clot retraction (see p. 30) is poor.
4. Red cells and hemoglobin are reduced in proportion to the hemorrhage.
5. Platelets are normal in number.

Vitamin-K Deficiency:

According to Clark and associates,[2] there are four factors necessary for adequate prothrombin formation: (1) a diet containing adequate vitamin K, (2) presence of bile in the intestinal tract, (3) normal intestinal mucosa for absorption, and (4) a liver capable of manufacturing prothrombin. Consequently, obstructive jaundice, biliary fistula, extensive pathological processes of the intestines (such as ulcerative colitis and polyposis), and liver damage are factors that may bring about a low prothrombin level and bleeding in adults.

Laboratory Examinations:

1. The prothrombin time is prolonged. The normal range for adults is ten to twelve seconds by the method of Quick. The prothrombin index is reduced.
2. The bleeding and coagulation times may be prolonged.
3. Clot retraction is poor.
4. Platelets are normal in number.

Hereditary Pseudohemophilia:

This is a nonthrombocytopenic hemorrhagic disease that is familial but is transmitted by either sex to either sex. Bleeding from the nose and mucous membranes is common, but cutaneous hemorrhages are rare.

Laboratory Examinations:

1. Bleeding time is prolonged, and coagulation time is normal.
2. Clot retraction may be delayed.
3. The platelet count is normal.

Severe Sepsis:

Infections, such as bacterial endocarditis, meningococcemia, typhoid fever, scarlet fever, septicemia, influenza, smallpox, measles, and diphtheria, may have associated purpuric manifestations. A fulminating purpura is that of epidemic cerebrospinal meningitis (Waterhouse-Friederichsen syndrome); it is adrenal hemorrhage, usually associated with meningococcemia. The platelet count may be decreased but usually shows no change. The bleeding-and-clotting time and prothrombin time should also be carried out to find out what clotting factor is deficient.

Liver Diseases:

Extensive liver damage, regardless of the cause, may reduce the capacity of the liver to produce an adequate amount of prothrombin or fibrinogen. Such damage may be the result of fatty degeneration, poisoning with chemicals that are toxic for the liver, and acute yellow atrophy.

Laboratory Examinations:

1. The prothrombin time is prolonged and the prothrombin index is reduced.
2. Clotting time is likely to be prolonged, and clot retraction is poor.
3. Bleeding time may be prolonged.
4. Platelet count is normal.

DEFICIENCY OF PLATELETS

Thrombocytopenic Purpura (Werlhof's Disease):

This is a purpuric disease of unknown cause, which is characterized by ecchymoses of the skin and hemorrhage from mucous membranes and into tissues. It is a disease of children and young adults, and is marked by relapses and remissions.

The tourniquet test is positive. This test is carried out by applying a blood-pressure cuff just above the elbow after noting any purpuric spots on the forearm. The cuff is inflated to a level between the systolic and diastolic pressures where it is maintained for five minutes. The appearance of petechial hemorrhages in the forearm indicates a positive reaction.

Laboratory Examinations:

1. The characteristic finding is a decrease in platelets (thrombocytopenia (see p. 28) or occasionally complete absence of platelets. Bleeding usually occurs when the platelet count is below 60,000 per cubic millimeter. However, in certain chronic forms of the disease, spontaneous bleeding may not occur even though the count is below 60,000.
2. There is variation in size and staining quality of platelets.
3. The bleeding time is prolonged as much as sixty minutes or longer. The clot does not retract. These findings, together with thrombocytopenia, are usually sufficient to confirm the diagnosis.
4. The coagulation time is within normal limits.

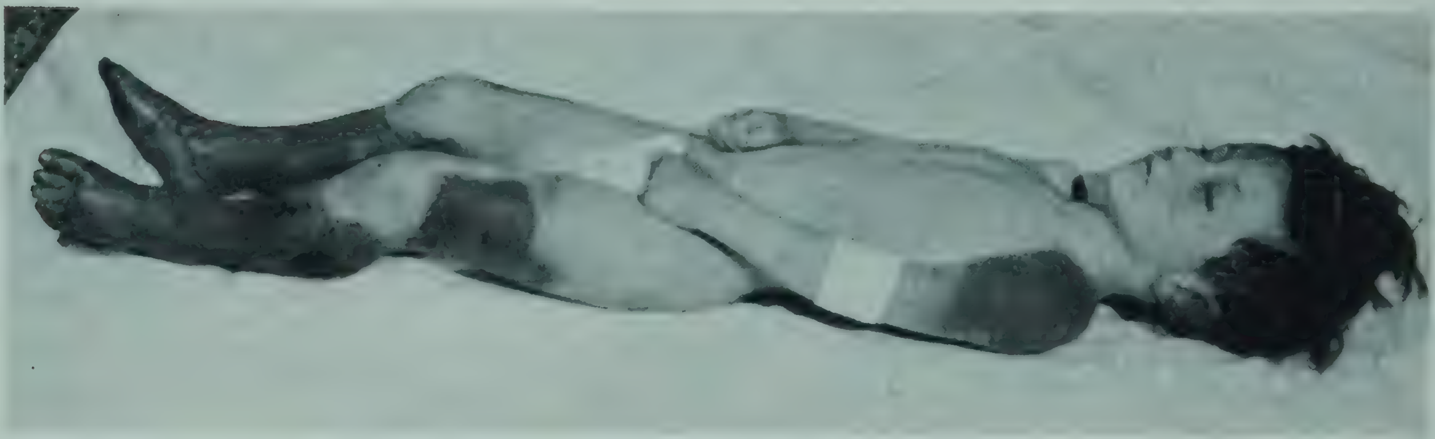

Fig. 1: Idiopathic thrombocytopenic purpura.

5. The red-cell count is decreased in proportion to the amount of hemorrhage, and the anemia is usually normocytic but may be hypochromic and microcytic or even macrocytic.
6. The leukocytes may be increased following a severe hemorrhage, but in the chronic disease, there is frequently a leukopenia. Sometimes the lymphocytes predominate.
7. Blood-fibrinogen, calcium, and prothrombin time are within normal limits.
8. According to Dameshek and Miller,[3] who studied five cases of idiopathic thrombocytopenic purpura, the megakaryocytes in the bone marrow ranged between 366 and 743 per million nucleated red cells as compared with not more than 300 in normal bone marrow. However, the production of platelets was greatly reduced, since only 8 to 19 per cent of the megakaryocytes showed evidence of breaking up in contrast to 68.6 per cent in normal persons. Following splenectomy in the purpura-hemorrhagica cases, 69 to 85 per cent of the megakaryocytes showed evidence of platelet formation.

Congenital Thrombocytopenia: This is a rare form of thrombocytopenic purpura. Both the mother and infant show a marked thrombocytopenia. However, bleeding and coagulation times remain normal, and purpura may not develop if the platelets show increased fragility.

Symptomatic Purpura: Symptomatic purpura is secondary to some disease or agent that injures the bone marrow and causes a thrombocytopenia. Aplastic anemia, myelophthisic anemia, leukemia, and sometimes pernicious anemia may be associated with thrombocytopenia, as well as other changes in the blood picture that characterize these diseases.

Thrombocytopenic purpura also occurs in splenic disorders, such as splenomegaly of undetermined origin (Banti's disease), Gaucher's disease, Niemann-Pick disease, and Hand-Schüller-Christian disease.

Physical agents, such as x-ray and radium, cause thrombocytopenia, but there is also an associated leukopenia and anemia. Toxins from burns may also cause a reduction in platelets.

Chemical agents, such as arsenic and benzene, venoms from snake and insect bites, and allergy to certain foods also cause thrombocytopenia in certain patients.

Thrombocytopenia with purpura is observed in some cases of subacute bacterial endocarditis, septicemia, typhoid fever, smallpox, vaccinia, scarlet fever, measles, miliary tuberculosis, and typhus fevers.

LABORATORY EXAMINATIONS: The same laboratory examinations are carried out as are listed under Thrombocytopenic Purpura (p. 362), and they have the same significance.

CAPILLARY FRAGILITY

Capillary fragility is chiefly an allergic manifestation, characterized by purpura plus erythema, urticaria, and so on. The purpuras of this group are the result of increased capillary permeability rather than bone-marrow disturbances with thrombocytopenia.

IDIOPATHIC

Hereditary Hemorrhagic Telangiectasia:

This is an hereditary hemorrhagic disease, which occurs in both sexes and is transmitted by both sexes. It is characterized by dilatation of capillaries in the skin and mucous membranes. The lesions are frequently present throughout childhood, but bleeding does not occur until adult life. Hemorrhage may occur from lesions in any location, but epistaxis is more common. The diagnosis is made clinically because laboratory examinations reveal only those changes secondary to hemorrhage.

SYMPTOMATIC

Allergic:

Nonthrombopenic Purpura (Henoch's Purpura):[4] This purpura occurs in children and adolescents. It is characterized by abdominal colic, which is central, vomiting, occasionally diarrhea with mucus and blood, and rheumatoid pains. Fever may be present but irregular. The abdominal symptoms occur before any purpuric spots develop, thus making the diagnosis more difficult. The symptoms are due to effusion into the intestinal wall. Laboratory examinations do not aid in the diagnosis.

Purpura Rheumatica (Schönlein's Purpura):[5] This disease occurs in young adults and is characterized by pain and tenderness around the joints or in the extremities. These symptoms are due to periarticular effusions. Purpura or other skin lesions may not occur until later. Fever plus joint pains suggest rheumatic fever, but there is no relief from salicylates.

Miscellaneous:

Vitamin-c deficiency (scurvy), certain infections, toxic drugs, chemicals, venenes, and chronic cardiorenal disease sometimes cause purpura because of damage to capillary endothelium. A miscellaneous group of purpuric diseases is found in hemochromatosis, purpura senilis, purpura cachetica, purpura simplex, purpura fulminans, and certain skin disorders.

LABORATORY EXAMINATIONS

Although no particular examination is diagnostic in the group of purpuras due to capillary fragility, the usual tests should be carried out as part of the program for establishing a diagnosis. They include platelet count, blood-cell studies, bleeding and coagulation times, clot retraction, prothrombin time, and the tourniquet test.

HEMOPHILIA

Hemophilia is a constitutional abnormality, which is transmitted by a sex-linked, recessive, mendelian trait. The disease is limited to males but is transmitted by unaffected females. For example, the daughter of a hemophiliac patient may transmit the disease to half of her sons.

The underlying defect in the clotting mechanism is not known, although various theories have been proposed. Minot and Lee[6] suggested that there is a qualitative defect in the platelets, and because of this, thromboplastic substance is formed more slowly than in normal blood. More recently, a lack of a globulin substance has been demonstrated in hemophiliac plasma by Howell.[7] The true nature of this substance is not known at present.

The disease is characterized by spontaneous hemorrhages or hemorrhages resulting from minor trauma. Hemorrhages occur from tooth sockets of loose teeth, and in the skin, muscles, kidneys, joints, mucous membranes, and peritoneal cavity. Bleeding out of all proportion to the injury occurs following minor wounds. The bleeding usually begins in childhood, sometimes as early as the first year.

Laboratory Examinations:

1. The characteristic finding is a prolonged coagulation time up to two hours or more.
2. Clot retraction is normal after the clot has formed.
3. Bleeding time is normal but may be prolonged during hemorrhage.
4. Prothrombin time and level are normal.
5. Platelets are normal in number or slightly increased.
6. Secondary anemia develops, depending on the amount of hemorrhage.
7. Moderate leukocytosis follows hemorrhage.
8. The tourniquet test is negative.

POLYCYTHEMIA

Erythremia:

This is also known as "polycythemia rubra," "polycythemia vera," or "Vaquez-Osler's disease." The etiology is unknown, and it occurs in both males and females. Hebrews are susceptible, but it is rare in Negroes. It manifests itself in the third and fourth decades after an insidious onset, but it has been described in children.

The patient has a ruddy red skin, various paresthesias and neurologic symptoms, splenomegaly, and a high red-cell count.

Laboratory Examinations:

1. The outstanding feature is an increased erythrocyte count ranging from 7,000,000 to 10,000,000 or more.
2. The volume of packed cells is increased, but the mean corpuscular volume is below normal.
3. Examination of the stained smear shows erythrocytes usually of normal size; but some variation in size, polychromatophilia, and basophilic stippling may be present.
4. Reticulocytes are usually normal in number.
5. Hemoglobin is increased and ranges from 18 to 24 gm. per 100 cc.
6. Leukocytes are increased with a shift to the left (15,000 to 25,000 per cubic millimeter).
7. Platelets are commonly increased and may reach 3,000,000 to 6,000,000 per cubic millimeter.[8]
8. The viscosity of the blood is increased, and the specific-gravity range is from 1.075 to 1.080 (normal: 1.055 to 1.065).
9. The bone marrow (see also p. 7) is hyperplastic with increased activity of all elements.
10. Bilirubinemia, which gives a positive, indirect, van den Bergh test is present.
11. The blood uric acid is increased in some cases.
12. The urine shows albuminuria and an increase of urobilinogen and urobilin.
13. The basal metabolic rate is increased to as high as +35.
14. There is an increase of urobilin in the feces.

Pseudopolycythemia:

This is symptomatic polycythemia, which is also known as "erythrocytosis." The underlying cause is the effect of anoxemia on the bone marrow, which results in the excessive production of erythrocytes. The anoxemia is caused by ascent to high altitudes where the atmospheric pressure is lower than at sea level. It is also caused by congenital and acquired heart disease, pulmonary emphysema, hyperplasia of the intima of the pulmonary artery (Ayerza's disease), and certain chemical agents and drugs that reduce the oxygen capacity of the blood. These include coal-tar derivatives, aniline and its derivatives, cobalt, manganese, mercury, iron, arsenic, bismuth subnitrate, digitalis, caffeine, and nicotine.

Laboratory Examinations: The results of laboratory examinations are similar to those under Erythemia, but in general the values are somewhat lower. For example, the red-cell count ranges from 7,000,000 to 9,000,000 per cubic millimeters, and the hemoglobin has a corresponding range.

LEUKEMIAS

Leukemia is a progressive disease, which is ultimately fatal. It is characterized by a widespread hyperplasia of the bone marrow, which is manifested by the discharge of large numbers of immature cells into the peripheral blood or fixed tissues, or both.

Etiology

Leukemia occurs in various animals and fowls, and in human beings of all races. Negroes are apparently less susceptible than whites. Hebrews have the chronic lymphocytic type of leukemia more frequently than other races, and for this reason appear to be more susceptible. The incidence of the disease is higher in the first five years of life than in any other five-year period. In the early years of life and through adolescence, it is likely to be the acute type.

The actual cause of leukemia in human beings is unknown, but leukemia of fowls can be transmitted by a cell-free Berkefeld filtrate. This makes the possibility of a virus etiology particularly intriguing. However, the disease does not spread from fowl to fowl as would be expected with a virus disease. Leukemia has been observed in persons exposed to x-ray, benzene, and aniline dyes. It might be inferred that exposure to these agents contributes to the development of the disease.

Classification

The classification of leukemias is at present based upon the predominating cell type. Certain leukemias are further classified as "acute" when immature cells are present and "chronic" when more mature cells predominate. With these facts in mind, leukemias may be classified as follows:

1. Myelogenous leukemia
    a. Acute
    b. Chronic

2. Eosinophilic leukemia (rare)
3. Basophilic leukemia (rare)
4. Lymphocytic leukemia
   a. Acute
   b. Chronic

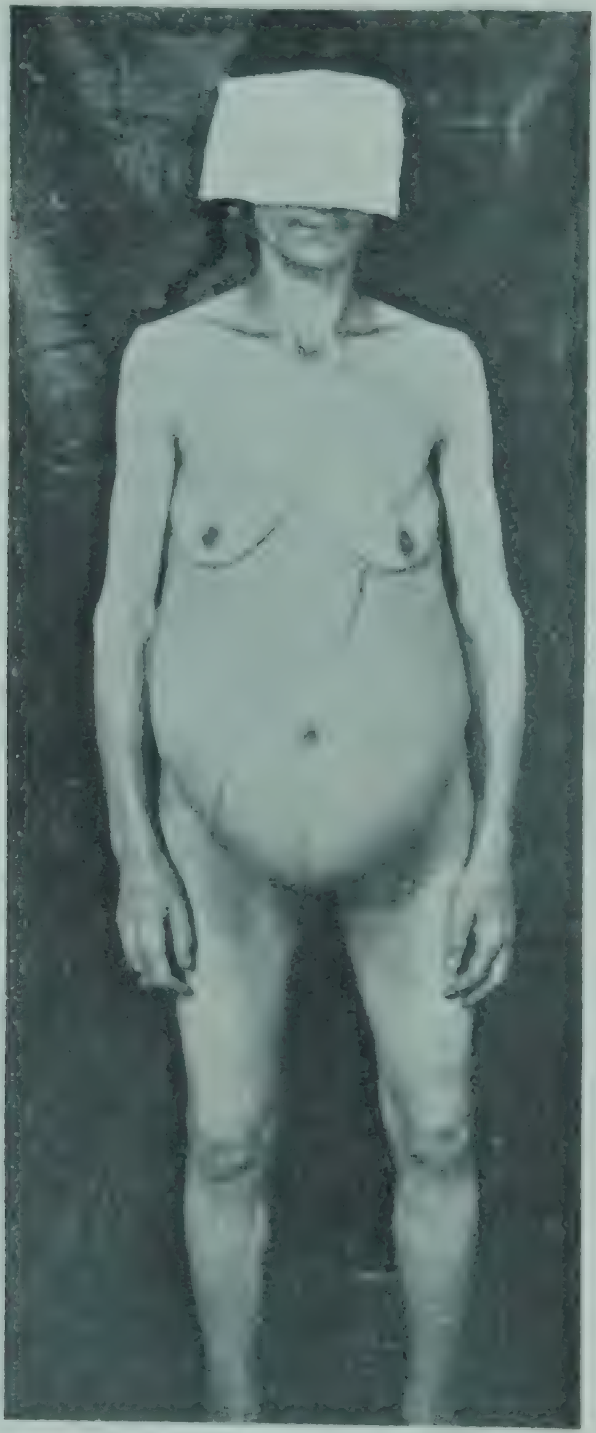

Fig. 2: Outline of enlarged spleen in patient with myelogenous leukemia.

5. Monocytic leukemia
6. Megakaryocytic leukemia (rare)
7. Plasma-cell leukemia (rare)

MYELOGENOUS LEUKEMIA (PLATE III)

Acute:

Clinically, acute myelogenous leukemia is characterized by pallor, weakness, anorexia, purpura, bleeding from the gums, joint pains, sore throat, and fever. Lymphadenopathy, particularly of the cervical group is usually present. Splenomegaly occurs in about 60 per cent of the cases. There may also be signs of heart failure, pulmonary congestion, ascites, edema, and enlarged liver. Nervous manifestations are facial paralysis, meningeal irritation, deafness, and intracranial hemorrhage.

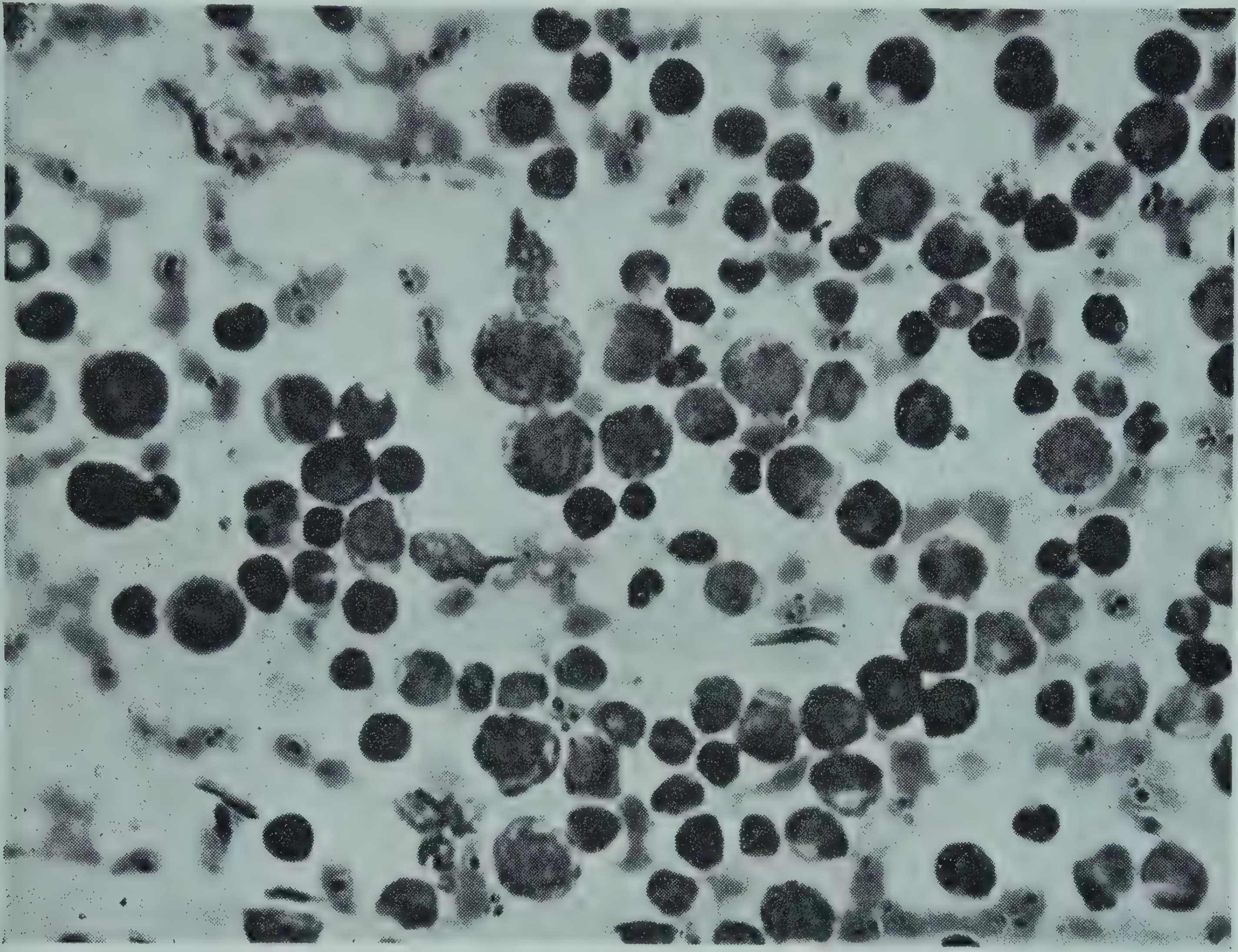

Fig. 3: Myelogenous leukemia.

Laboratory Examinations:

1. The characteristic finding is a high white-cell count. The average range is 50,000 to 100,000 per cubic millimeter. However, in the aleukemic phase, the total leukocytes may be as low as 400 per cubic millimeter.
2. Examination of the stained smear is necessary to determine the type of leukemia. Acute myelogenous leukemia is characterized by a preponderance of myeloblasts, promyelocytes, and myelocytes. Adult neutrophils are few, and eosinophils and basophils are not usually present. Auer's bodies are present in about 25 per cent of the cells. These are rod-shaped structures in the

cytoplasm, which are peroxidase-positive and may be present in myeloblasts, myelocytes, and monocytes. Lymphocytes and monocytes are fewer than in normal smears.

3. The red-cell count is reduced, and the range is from 3,000,000 to as low as 1,000,000 or less. The hemoglobin is correspondingly reduced. The cells are normocytic or sometimes macrocytic. Normoblasts and polychromatophilic cells are present.
4. Platelets are reduced usually below 100,000 per cubic millimeter. Large platelets with bizarre shapes are frequently seen.
5. Bleeding time and sometimes the coagulation time are prolonged.
6. Clot retraction is poor.
7. The tourniquet test is usually positive.
8. The bone marrow shows a predominance of young cells of the neutrophilic series, particularly myeloblasts. These cells crowd out the erythropoietic cells and thus contribute to the anemia (see p. 7).

Chronic:

The onset of chronic myelogenous leukemia is insidious and may be discovered only accidentally through a blood examination. The patient may have anemia, loss of weight, anorexia, flatulence, purpura, or Vincent's angina. In more advanced cases, the anemia is more marked, and the lymph glands, spleen, and liver are enlarged. The patient becomes emaciated, the heart is enlarged, and a systolic murmur may be present. Herpes zoster and leukemia cutis are sometimes seen in advanced cases.

Hematuria and priapism may be present, particularly in those with high white counts. Females may experience menorrhagia, metrorrhagia, or amenorrhea.

Laboratory Examinations:

1. The leukocyte count ranges from 100,000 to 800,000 per cubic millimeter. Low counts may be found occasionally, and in such cases a careful study of the stained smear is necessary to diagnose leukemia in the aleukemic phase.
2. Examination of the stained smear reveals 35 to 70 per cent of adult and nonsegmented neutrophils. Myelocytes make up 20 to 40 per cent; and myeloblasts, 2 to 12 per cent. Eosinophilic and basophilic myelocytes are found. In fact, adult eosinophils and basophils are sometimes increased. Lymphocytes and monocytes are decreased, particularly late in the disease.

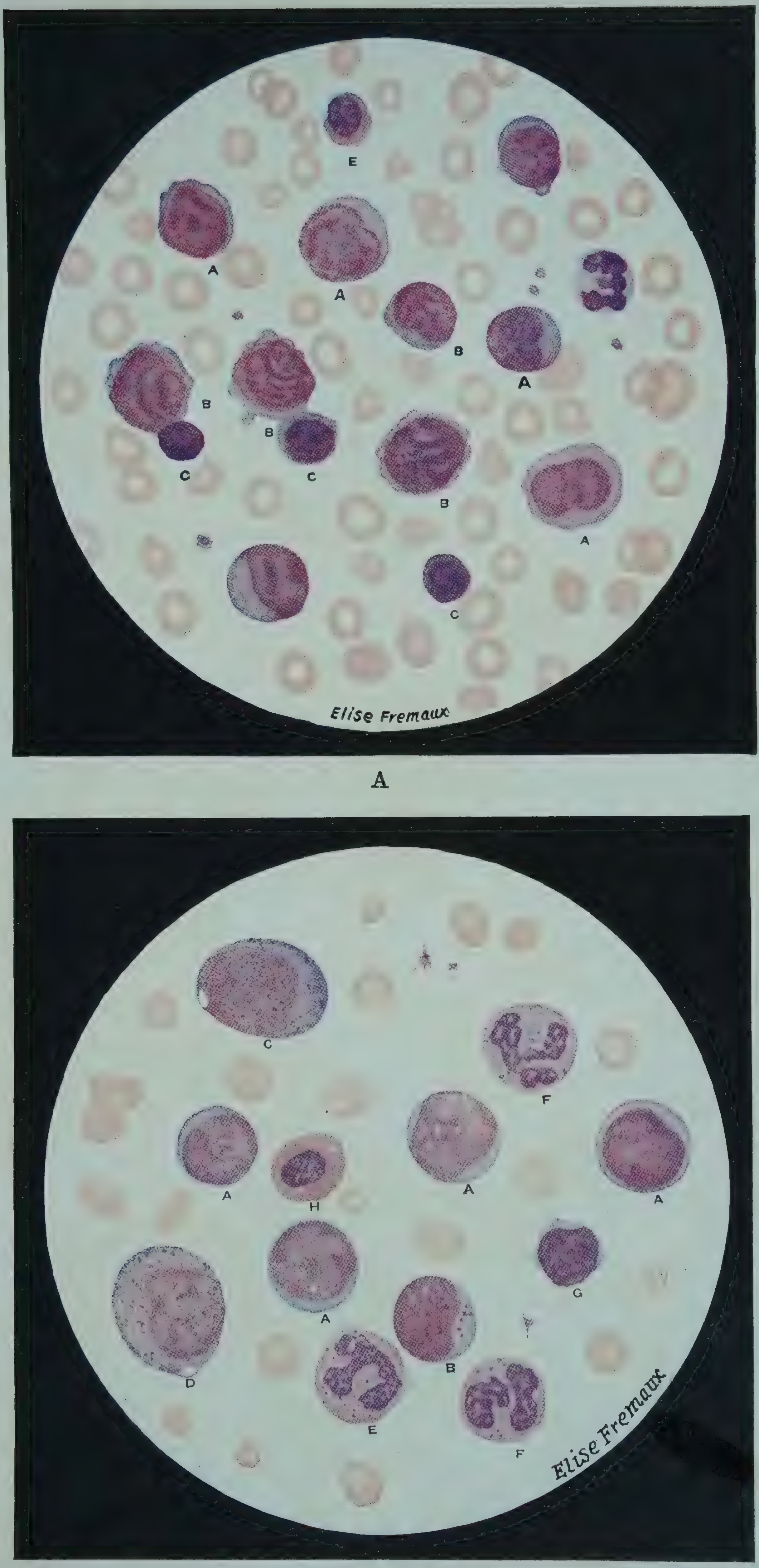
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Plate III: *A*: Acute lymphoblastic leukemia. *B*: Acute myeloblastic leukemia. (From Tice: Practice of Medicine. W. F. Prior Co., Hagerstown, Md.)

3. A well-marked anemia (from 3,000,000 to less than 2,000,000 per cubic millimeter) is present when the patient presents himself for examination. The cells are normocytic. Variation in size is marked, but variation in shape is not usually present. Normoblasts, polychromatophilic cells, and stippled cells are seen. Reticulocytes are increased.
4. Platelets are usually normal in number. When the count falls and remains low, the outlook is grave.
5. Bleeding and coagulation times are not changed while the platelets remain normal.
6. The bone marrow is hyperplastic, and most of the cells are immature forms of the neutrophilic series.

EOSINOPHILIC LEUKEMIA

This rare disease is characterized by lymphadenopathy, splenomegaly, hepatomegaly, and a leukocytosis with anemia.

Laboratory Examinations:

1. The leukocyte count is increased to 250,000 or more per cubic millimeter. Although eosinophilic myelocytes are present, the adult forms predominate. The eosinophils usually total 80 to 90 per cent.
2. There is a moderate anemia with a red-cell count of about 3,000,000. The cells are normocytic, and normoblasts are present. With more marked anemia, polychromatophilia and stippled cells may be seen.

BASOPHILIC LEUKEMIA

This disease is also rare, but the clinical picture may simulate either chronic or acute myelogenous leukemia. A leukocytosis and anemia are present, but the smear shows a marked increase in basophils. A case reported by Groat and co-workers[9] had 24 to 61 per cent of basophils, and it simulated acute myelogenous leukemia.

LYMPHOCYTIC LEUKEMIA (PLATE III)

Acute:

The symptoms and signs of acute lymphocytic leukemia are similar to those of acute myelogenous leukemia. Generalized lymphadenopathy and splenomegaly are more likely to be found in this form of leukemia.

Laboratory Examinations:

1. The white-cell count is characteristically increased to from 50,000 to 100,000 per cubic millimeter.
2. The predominating cells in the stained smear are those of the lymphocytic series including lymphoblasts and mature lymphocytes. The lymphoblasts make up from 50 to 90 per cent of the white cells and are characterized by round or oval nuclei, which have coarse granular chromatin and one or two nucleoli. The chromatin appears to be more compact around the edges of the nuclei, thus suggesting a membrane. There may be a clear zone in the cytoplasm around the nuclei. The more immature forms are difficult to differentiate from myeloblasts. The cytoplasm is basophilic but is of a lighter shade than the nucleus. No granules are present. Very few granulocytes are seen, and they are usually neutrophils.

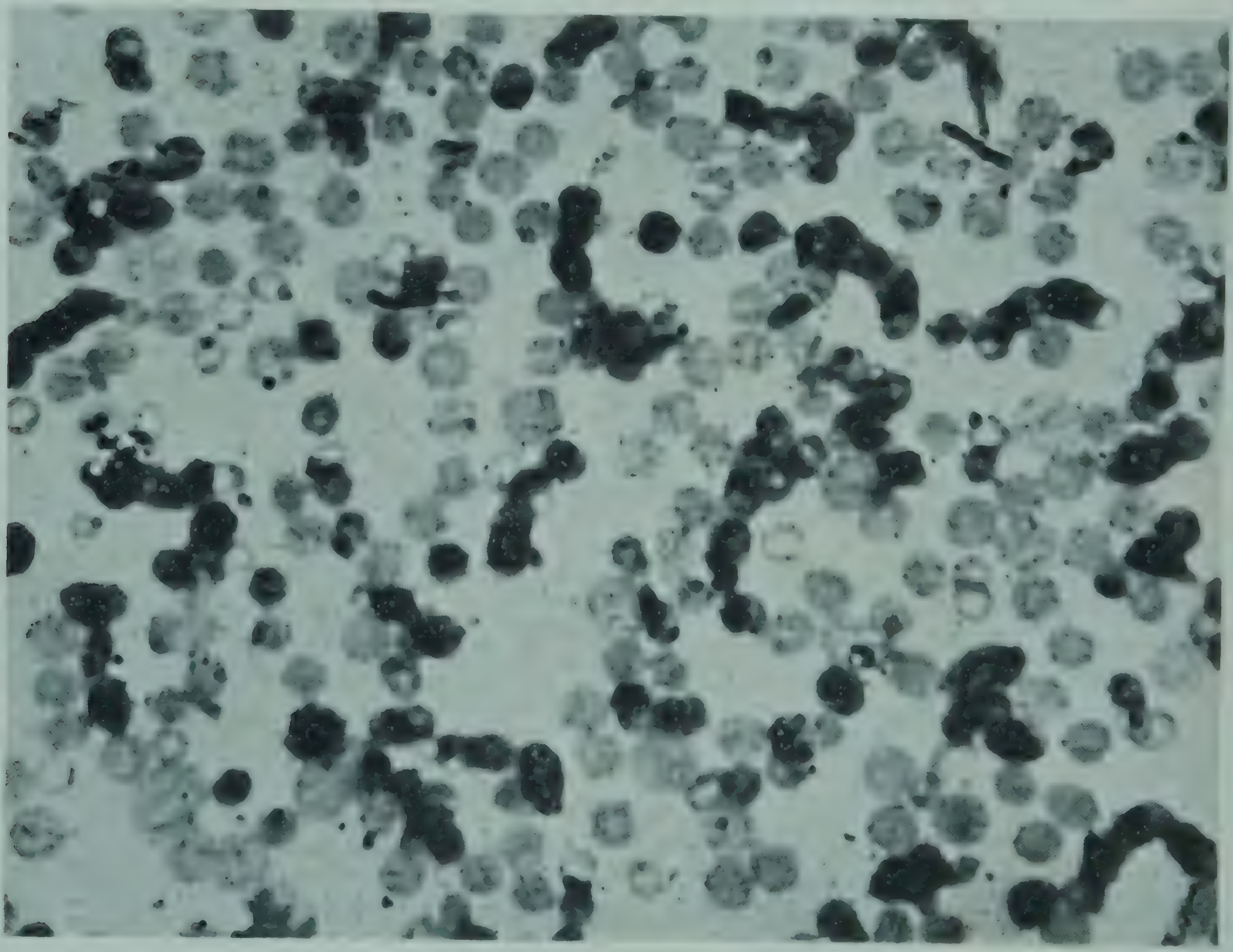

Fig. 4: Chronic lymphocytic leukemia.

3. The red-cell count is reduced. In the more advanced cases, the anemia is severe, and the erythrocytes may be as low as 1,000,000 per cubic millimeter. The red cells are normocytic, and normoblasts and polychromatophilic cells are present.

4. Platelets are usually reduced below 100,000 per cubic millimeter, and giant forms are seen. If the platelets go below 60,000, the bleeding time is prolonged and clot retraction is poor.
5. For bone-marrow changes, see p. 7.

Chronic:

There is very little difference in the signs and symptoms of the chronic forms of the different types of leukemia. In chronic lymphocytic leukemia, however, adenopathy appears early and may be extensive. The spleen is only moderately enlarged and is usually not so large as that in chronic myelogenous leukemia. Various types of skin lesions are more common in this type of leukemia.

Laboratory Examinations:

1. The leukocyte count is usually lower than in myelogenous leukemia. It is rarely higher than 250,000 per cubic millimeter.
2. The differential count has more than 90 per cent of small lymphocytes. The fields have a monotonous appearance because of the uniformity of cell type. A few immature lymphocytes are found, but in general the process does not appear to be so active as the acute type.
3. Reduction of erythrocytes and platelets is usually present. The anemia and hemorrhage potential are present in proportion to the decrease in these elements.
4. For bone-marrow changes, see p. 7.

MONOCYTIC LEUKEMIA

The majority of cases run an acute course, while a few (11 per cent) are chronic. Clinically, the disease may be divided into acute and chronic, but the microscopic examination does not differentiate the two types.

Laboratory Examinations:

1. The leukocyte count is increased but is usually less than 100,000 per cubic millimeter.
2. Examination of the stained smear shows large cells, which have the appearance of monocytes and constitute 60 per cent or more of the cells in the differential count. The remaining cells are made up of lymphocytes, polymorphonuclear leukocytes, and sometimes myeloblasts, myelocytes, and plasma cells.

MEGAKARYOCYTIC LEUKEMIA

In this disease, the bloodstream is flooded with megakaryocytes and atypical platelets. The leukocyte count is normal or subnormal. In the smear, young megakaryocytes and atypical platelets stand out. Normoblasts and polychromatophilic cells are also observed.

Some cases reported under this heading are not considered megakaryocytic leukemia but are probably acute myeloblastic leukemia with hyperplasia of megakaryocytes.

PLASMA-CELL LEUKEMIA

This disease has the signs and symptoms of leukemia with immature plasma cells predominating in the smear.

Laboratory Examinations:

1. The leukocyte count is increased up to 60,000 per cubic millimeter.
2. The stained smear contains immature plasma cells, which predominate over the other cells.
3. There is a moderate normocytic anemia.
4. There are deposits of plasma cells in the lymph nodes, liver, spleen, and bone marrow.

ALEUKEMIC LEUKEMIA

Leukemia of this type is difficult to diagnose because of a normal or subnormal, total leukocyte count. Examination of the blood smear reveals a few myeloblasts and myelocytes, which are usually of the neutrophilic series. However, lymphocytic and monocytic leukemias have been reported in the aleukemic phase, and it is possible to differentiate them into acute and chronic forms. There is frequently a terminal rise in the leukocyte count which helps to make the diagnosis. Examination of bone-marrow smears (see also p. 8) is helpful in making a diagnosis. According to Mettier and Purviance,[10] 10 per cent or more of leukemia cases are detected in the aleukemic phase.

LEUKEMOID REACTIONS

Leukemoid reactions are those changes in the blood picture that make it resemble leukemia. Such changes in the blood are seen particularly in children with severe sepsis, as in extensive lobar pneumonia. They are sometimes seen also in the bone-marrow crisis of hemolytic jaundice and sickle-cell anemia, syphilis, acute tuberculosis, meningitis, osteomyelitis, von Jaksch's anemia and familial erythroblastic anemia (Cooley's anemia), infectious mononucleosis, pertussis, metastatic tumors in the bone marrow, malignant granuloma (Hodgkin's disease), whooping cough, and pyelonephritis.

Laboratory Examinations:

1. The total leukocyte count is increased up to 100,000 or more per cubic millimeter.
2. Examination of the smear shows myeloblasts of not over 10 per cent, while in leukemia they may number 90 per cent or more.
3. There is no appreciable anemia unless the patient has an anemia from some other cause. In leukemia, there is a progressive normocytic anemia.
4. Platelets are usually normal. They are usually deceased in leukemia, although they may be normal or increased in chronic myelogenous leukemia.

AGRANULOCYTOSIS

Agranulocytosis is a prostrating disease due to various causes, chiefly drugs, and characterized by a marked reduction in granulocytes. It appears to result chiefly from the consumption of drugs containing aminopyrine. It is also associated with benzene, arsenicals, sulfonamides, gold salts, and dinitrophenol. Sepsis has also been considered a cause, but it has not been universally accepted.

Clinically, it is characterized by sudden onset, chills, high temperature, headache, and gangrenous ulcerations in the mouth or pharynx. Many cases end fatally because of the overwhelming infection which the body is unable to combat.

Laboratory Examinations:

1. The outstanding feature is a low leukocyte count, ranging from 2000 to as low as 100 per cubic millimeter. In chronic nonfulminating cases, the count may range from 4000 to 2000 per cubic millimeter.
2. The smear shows either complete absence of granulocytes or only 1 to 2 per cent. Lymphocytes and monocytes make up almost the entire differential count. In chronic cases, granulocytes are not reduced to such a degree, and a few myelocytes may be present.

3. The erythrocytes and platelets are not reduced in the average case.
4. The sedimentation rate is increased.
5. Examination of the bone marrow (see also p. 8) shows a lack of granulocytes, both adult and immature. The erythropoietic cells and megakaryocytes are present in normal numbers.

EFFECTS OF ATOMIC-BOMB EXPLOSION

The effects of atomic-bomb explosion fall into three groups: (1) injuries from the blast, including injuries and burns from falling and flying debris; (2) flash burns; and (3) radiation effects. This section is concerned with radiation effects because of the pathological changes that take place in the blood and bone marrow.

Radiation Sickness:

The clinical result of excessive radiation by gamma rays is known as "radiation sickness." In the explosion of the atomic bomb, the gamma rays that cause the radiation sickness include neutrons and other penetrating radiation liberated by the fission process and produced as part of the total spectrum of energy.

The following description of the clinical picture of radiation sickness is taken from The United States Strategic Bombing Survey:[11]

Clinical Picture:

In general, the gamma rays were very penetrating. There were no skin lesions which could be directly attributed to gamma rays even in those patients showing severe radiation disease. The Japanese stated that those individuals who were very near the center of the explosion but did not suffer from flash burns or secondary injuries, became ill within two or three days. Hemorrhagic diarrhea followed and they expired quite quickly. Some died within two or three days after the onset and most of them within a week. The Japanese reported that it was difficult to demonstrate the remarkable changes in the blood picture of those dying so rapidly. Upper respiratory and gastrointestinal mucous membranes showed acute inflammation.

The larger portion of the radiation cases which had been at greater distances did not show severe symptoms until one to four weeks after the explosion. Many felt weak and listless on the day following the bomb burst. Nausea and vomiting appear also to have been frequent at this time. Within a day or two, the appetite improved and the person felt quite well until symptoms appeared at a later date. It is the opinion of some of the Japanese physicians that those who rested and subjected themselves to less physical exertion showed a longer delay before the onset of subsequent symptoms. After this period during which the individuals usually felt perfectly well, they began to note lassitude, malaise, and anorexia. Within twelve to forty-eight hours fever became evident. In many instances it reached only (102.2° F.) (39° C.) and remained for only a few days. With the subsidence of fever, the patients usually showed a rapid disappearance of other symptoms and soon regained their feeling of good health. The degree of fever apparently had a direct relationship to the degree of exposure to radiation. In many other cases, the temperature was quite high, reaching 104° F. (40° C.) to 105.8° F. (41° C.). The fever once developed was usually well sustained, and in those cases terminating fatally, it continued high until the end. Other manifestations which were commonly seen were leukopenia, epilation, inflammation and necrosis of the gums, stomatitis, pharyngitis, petechiae, and evidence of ulceration of the lower gastrointestinal tract.

Leukopenia appears to have been a constant accompaniment of radiation disease. It is believed that leukopenia existed in some of the milder cases without other evidence of radiation effects. It is also thought that the degree of leukopenia was probably the most accurate index of the amount of radiation the person received. The white-blood count in the more severe cases ranged from 1500 to 0 with a complete absence of neutrophils. The moderately severe cases showed total white-blood counts of 1500 to 3000 with a neutropenia, and the bone marrow showed evidence of degeneration. The milder cases showed white-blood counts of 3000 to 4000 with minor degenerative changes in the bone marrow. The changes in the erythropoietic system were usually not as startling but corresponded roughly to the deficiency of white-blood-cell formation. Blood-platelet formation also was reported to be affected.

Epilation usually began about two weeks after the bomb explosion, though in a few instances it is reported to have begun as early as four to five days after the exposures. The areas involved were affected usually in the following order, depending upon the degree of exposure to radiation; scalp, axilla, beard, pubic region, and eyebrows. The involvement of the scalp was usually uniform and rarely resulted in complete baldness. Microscopic section of the involved areas has shown atrophy of the hair follicles. An interesting feature of the epilation, which has been reported by the Japanese and has not been confirmed, is that it appeared to be less marked in persons with gray hair than in persons with dark hair even though the exposure was the same. In those patients who survived two months, evidence of regeneration of the hair was present almost without exception.

Ulceration of the gastrointestinal tract, including gingivitis, stomatitis, and pharyngitis, was common in the more severe cases. The clinical picture seen in these cases is essentially the same as that seen in leukopenias due to other causes. Bloody diarrhea was commonly seen but in many instances was difficult to evaluate because of the possibility of its having been on an infectious basis. Petechiae occasionally appeared in the mucous membranes about the time of the onset of the gingivitis. However, those hemorrhages appearing in the skin were often more delayed.

Following is a summary of history and laboratory findings in the case of a twenty-four-year-old female who recovered:

History: She was in a Japanese house 1200 meters from center of blast and was knocked unconscious by falling ceiling. She remained well until about September 1 when she noted weakness, malaise, and soreness of gums. Generalized epilation of scalp had begun on August 16; pharyngitis, on September 3; generalized petechiae, on September 4; and bloody diarrhea on September 5 only.

Physical examination showed epilation of scalp, necrotic gingivitis, bilateral exudative pharyngitis, generalized petechiae, fever, and pallor.

Laboratory Findings:

September	*Red-Blood Count*	*Hemoglobin Per Cent*	*White-Blood Count*	*Miscellaneous*
5........	2.43	28	480	
6........	....	..	450	Bleeding time = 30 minutes Clotting time = 3½ minutes
7........	....	..	320	Stool (occult blood = 0); urine (albumin ±)
9........	....	..	240	Urobilin = 0
10........	....	..	170	Urobilinogen = 0
11........	1.76	35	240	
13........	....	..	980	
14........	2.11	40	1860	
15........	....	..	2260	
17........	....	..	3400	Sedimentation rate = 52 (Westergren)
18........	2.40	45	3840	
26........	3.07	56	4740	Eosinophils = 1; juvenile forms = 3; stab forms = 11; segmented polymorphonuclear leukocytes = 50; lymphocytes = 28; monocytes = 4

Keller[12] had an opportunity to examine victims of the atomic explosion. His findings are summarized as follows:

White-blood cells showed a leukopenia of from 1500 to 0 with a decrease or absence of neutrophils. Red-blood cells showed a moderate hypochromic microcytic and hypochromic normocytic anemia. Platelets were decreased. Sedimentation rate was markedly increased in most cases. One rate was as high as 144 mm. in the first hour and 148 mm. in the second hour by the Westergren technic. The bleeding time was prolonged; the average by the Duke method was nine minutes. The clotting time and fragility were normal.

INFECTIOUS MONONUCLEOSIS

Infectious mononucleosis (see also p. 104) is an infectious disease characterized by lymphadenopathy, sore throat, fever, and sometimes by prostration, splenomegaly, and a Vincent's infection of the throat. The cause is not known, but a virus etiology has been suggested by Wising,[13] who claims to have transmitted the disease to monkeys. It occurs mostly in children and young adults, and males appear to be more susceptible than females.

Laboratory Examinations:

1. The white-cell count is increased to from 15,000 to 25,000, but during the first week there may be a leukopenia.
2. Examination of the blood smear shows 60 per cent or more of the cells of the nongranulocytic series. Among these, there may be 10 to 25 per cent of atypical large lymphocytes, characterized by a dull blue cytoplasm containing one or more fenestrations. The smear also contains small lymphocytes and monocytes.
3. There is sometimes a decrease in platelets with a prolonged bleeding time and normal coagulation time.
4. Examination for heterophil agglutinins (the Paul-Bunnell test[14]), is helpful in making a diagnosis. The agglutinins in normal human serum will agglutinate sheep cells in a dilution usually not higher than 1:8. In infectious mononucleosis, agglutination takes place in dilutions of 1:32 or higher. An increasing titer in conjunction with lymphadenopathy and leukocytosis is particularly significant.
5. False-positive serological tests for syphilis have been found in about one fifth of the cases.[15] These are positive temporarily only if the person does not have syphilis.

6. Examination of the cerebrospinal fluid may show a moderate increase in pressure, an increased cell count with lymphocytes predominating,[16] or a normal cell count. The total protein may be increased, and associated with this the Pandy test is likely to be positive.
7. For bone-marrow findings, see p. 8.
8. Differential test: The antisheep-cell agglutinins are not of the Forssman type and are not absorbed by a suspension of guinea-pig kidney. The antibodies in horse-serum sickness are of the Forssman type and are readily absorbed by guinea-pig kidney. The differential test with guinea-pig antigen as suggested by Davidsohn and Walker,[17] is therefore indicated in the following: (1) cases following injection of horse serum which have a sheep-cell, antibody titer (heterophilic agglutinins) of 1:224 or more; (2) cases without clinical or laboratory evidence of the disease with a sheep-cell, antibody titer of 1:224 or higher; and (3) cases with a low sheep-cell, antibody titer which are clinically suggestive.

LYMPHOBLASTOMA

Laboratory tests with the exception of a biopsy are of limited value in the diagnosis of lymphoblastoma. The following changes are found in malignant granuloma (Hodgkin's disease) with variations in other lymphoblastomas as indicated.

1. There is a normocytic anemia with a red count sometimes as low as 1,500,000 per cubic millimeter. In lymphosarcoma and follicular lymphoma, the red count is usually above 4,000,000, particularly early in the disease.
2. The white-cell count varies from a leukopenia to a leukocytosis. This is also true for other forms of lymphoblastoma.
3. The differential count reveals an eosinophilia, lymphocytopenia, monocytosis, and frequently a neutrophilia. However, when the bone marrow is invaded, there is a decrease in neutrophils.

   Leukemialike smears are sometimes seen in association with lymphocytic or lymphoblastic lymphomas. Such a picture sometimes develops after the diagnosis has been made from a lymph gland.
4. The platelets may be increased or normal in malignant granuloma. When the platelets are decreased, it suggests bone-marrow involvement.
5. Bone-marrow examinations may reveal no changes; but if there are any, they reflect those in the blood. There may be a slight

shift to the left in the neutrophilic series. In one of our cases, Reed-Sternberg cells were found in the sternal bone marrow.

6. The basal metabolic rate is increased.
7. When the bones are involved, there is an increase in alkaline phosphatase.
8. The sedimentation rate is normal, but may be increased when the disease is active.
9. The uric-acid level may be increased.

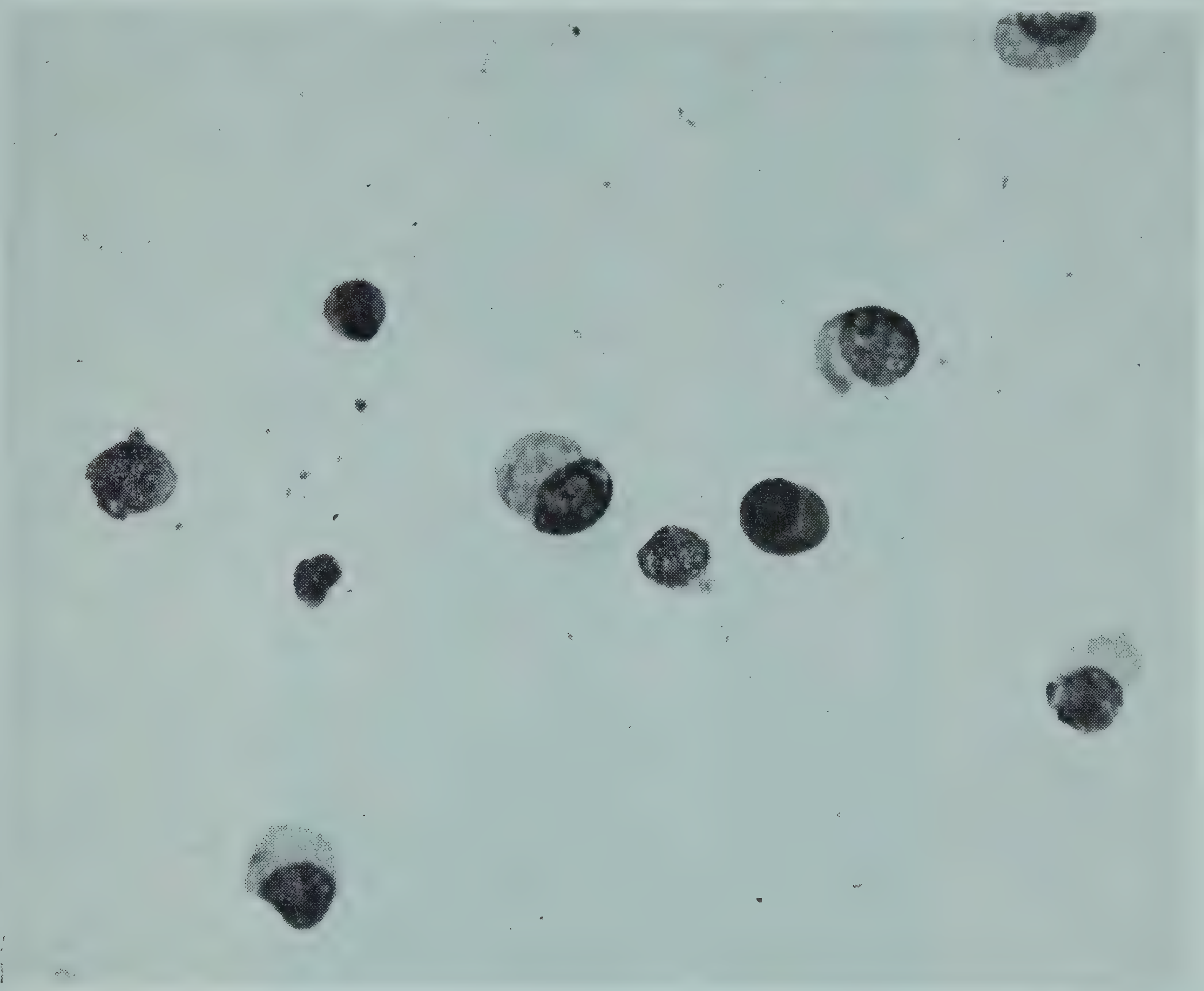

Fig. 5: Plasma cells in bone-marrow smear from case of plasma-cell myeloma.

MULTIPLE MYELOMA

Multiple myeloma is a tumor usually of the bone marrow, which causes pain, anemia, and pathological fractures, and frequently produces a characteristic protein (Bence-Jones protein) in the urine.

Laboratory Examinations:

1. Smears or sections of bone marrow that contain myeloma cells[18] are diagnostic. These cells may make up 3 to 65 per cent of

the total number in a differential count of the bone marrow. Although myeloma cells suggest plasma cells, they do not have the wheel-spoke arrangement of the nuclear chromatin, and the cytoplasm is bright blue instead of blue green.

2. Bence-Jones proteinuria is observed in about 65 per cent of cases. According to Geschichter and Copeland,[19] it may be intermittent early in the disease.
3. The urine may also contain albumin and casts, and the specific gravity may be low. Excretion of phenolsulphonphthalein may be decreased, and nitrogen retention may take place.
4. There is usually a moderate anemia with erythrocyte counts ranging from 4,000,000 to 3,000,000 per cubic millimeter. Normoblasts, polychromatophilic cells, and stippled cells may be observed. It has been noted that erythrocytes clump in the counting chamber, and counting the cells is thus rendered difficult. The difficulty can be overcome by diluting with normal saline or by heating the usual diluent. There is also marked rouleaux formation in the moist smear.
5. The leukocyte count is usually within normal range. Among the abnormalities in the differential count may be noted occasional myelocytes and myeloblasts, or an increase in eosinophils. Sometimes, myeloma cells or plasma cells are seen.
6. The platelet count is not usually remarkable, but it has been decreased in some cases.
7. Hyperproteinemia is characteristic. It is frequently 10 gm. per 100 cc. or even more. The increase is due to an increase in the globin fraction. The formol-gel reaction is positive.[20]
8. The serum calcium is frequently increased, and the inorganic phosphorus is normal or increased.

PARASITIC DISEASES

Malaria:

Four species of Plasmodia cause malaria in human beings, namely Plasmodium vivax (tertian), Plasmodium malariae (quartan), Plasmodium falciparum (estivoautumnal), and a rare tertian form, Plasmodium ovale.

Laboratory Examinations: The diagnosis is made by finding some stage of the infecting Plasmodium in the blood smear. Both thick and thin smears should be prepared and examined in order to increase the chances of finding the parasites. They are most numerous during the latter part of a febrile seizure and shortly afterward.

Filariasis:

Filariasis is usually caused by Wuchereria bancrofti, but it may be caused by other species, such as Loa loa, Wuchereria malayi, Mansonella ozzardi, Onchocerca volvulus, and Acanthocheilonema perstans.

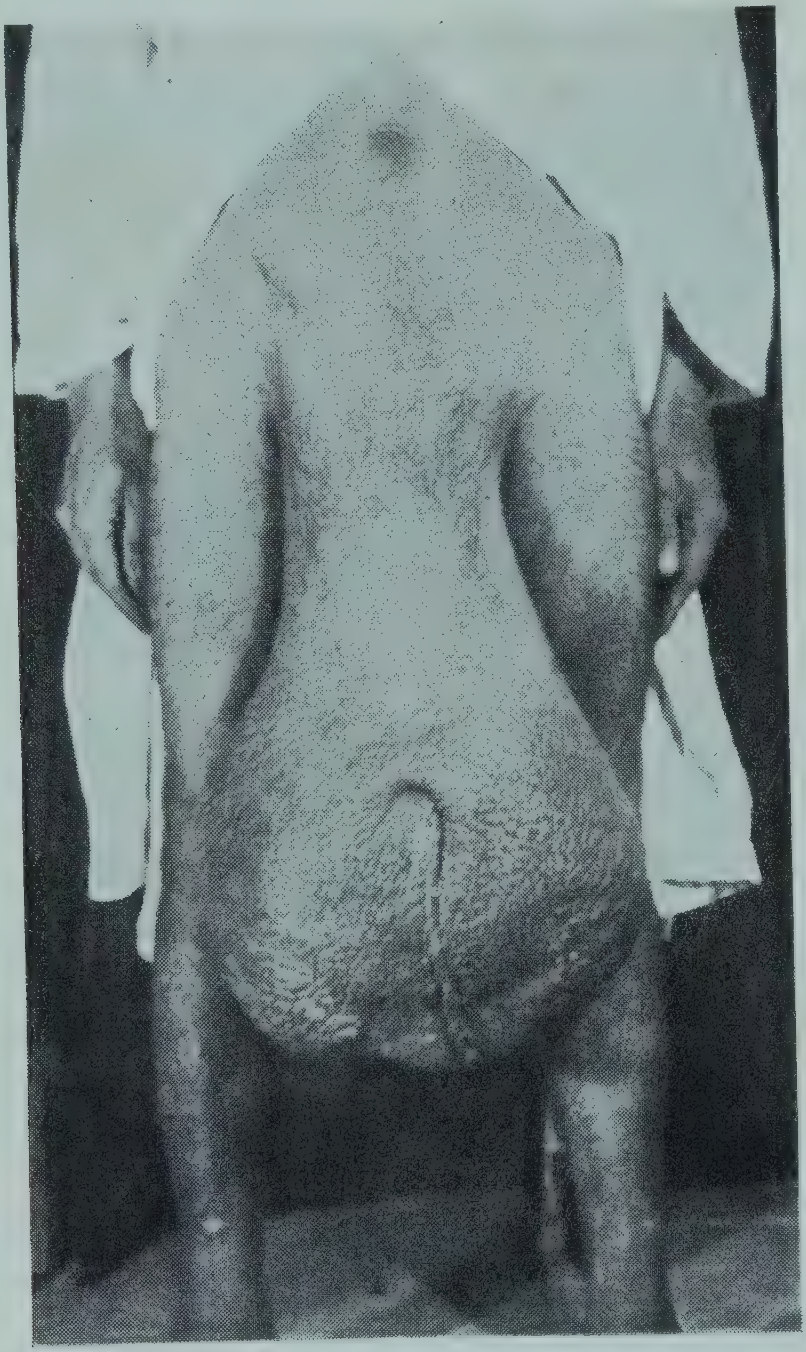

Fig. 6: Elephantiasis of scrotum caused by Wuchereria bancrofti.

The common form of the disease, after repeated infections, is characterized by elephantiasis, which is a result of lymphangitis with obstruction of the lymphatics, lymphadenopathy, hydrocele with fibrotic thickening of the scrotum, and sometimes chyluria.

Laboratory Examinations:

1. Thick, wet or stained blood smears are taken between 10 P.M. and 2 A.M. in those cases that may be infested with filaria which

have nocturnal periodicity. Loa loa microfilariae may be found in the blood during the day.
2. Skin tests and complement-fixation tests may be carried out with aqueous or alcoholic extracts of Dirofilaria immitis. A negative test is more significant than a positive test. A positive test is not indicative of infestion with a specific filaria. It may be interpreted only as infestation with some parasite of the filaria group.

Trypanosomiasis:

This is also called "sleeping sickness" and "African trypanosomiasis." It is caused by Trypanosoma gambiense or Trypanosoma rhodesiense. American trypanosomiasis (Chaga's disease) is caused by Trypanosoma cruzi.

Laboratory Examinations:

1. Examination of wet blood films and stained thick and thin smears reveals the trypanosomes, especially during bouts of fever.
2. Material aspirated from lymph glands may contain the trypanosomes.
3. In the later stages of the disease, examination of the centrifuged sediment of the cerebrospinal fluid is sometimes helpful in establishing a diagnosis.
4. The laboratory diagnosis of American trypanosomiasis is usually unsuccessful except during febrile exacerbations in chronically ill adults and during the febrile periods in children. If the smears are negative, the blood should be inoculated into a guinea pig. Complement-fixation tests with antigen made from Trypanosoniasis cruzi are sometimes useful in establishing a diagnosis.

Leishmaniasis:

Visceral leishmaniasis is also called "kala-azar" and is caused by Leishmania donovani. This is different clinically from oriental sore, or cutaneous leishmaniasis, which is caused by Leishmania tropica, and American leishmaniasis, or espundia, which is caused by Leishmania braziliensis.

Laboratory Examinations:

1. Thick smears of the peripheral blood sometimes show the Leishmania donovani in the polymorphonuclear leukocytes and monocytes.

2. Examination of smears and inoculation of hamsters with material from splenic puncture is usually a more successful way of making the diagnosis. Smears or sections from enlarged lymphatic glands are also helpful in demonstrating the parasite. The parasite can also be cultured on N.N.N. (Nicolle, Navy, McNeal) medium.
3. Smears from the skin lesions of cutaneous leishmaniasis show the Leishmania-tropica parasites within the histiocytes.

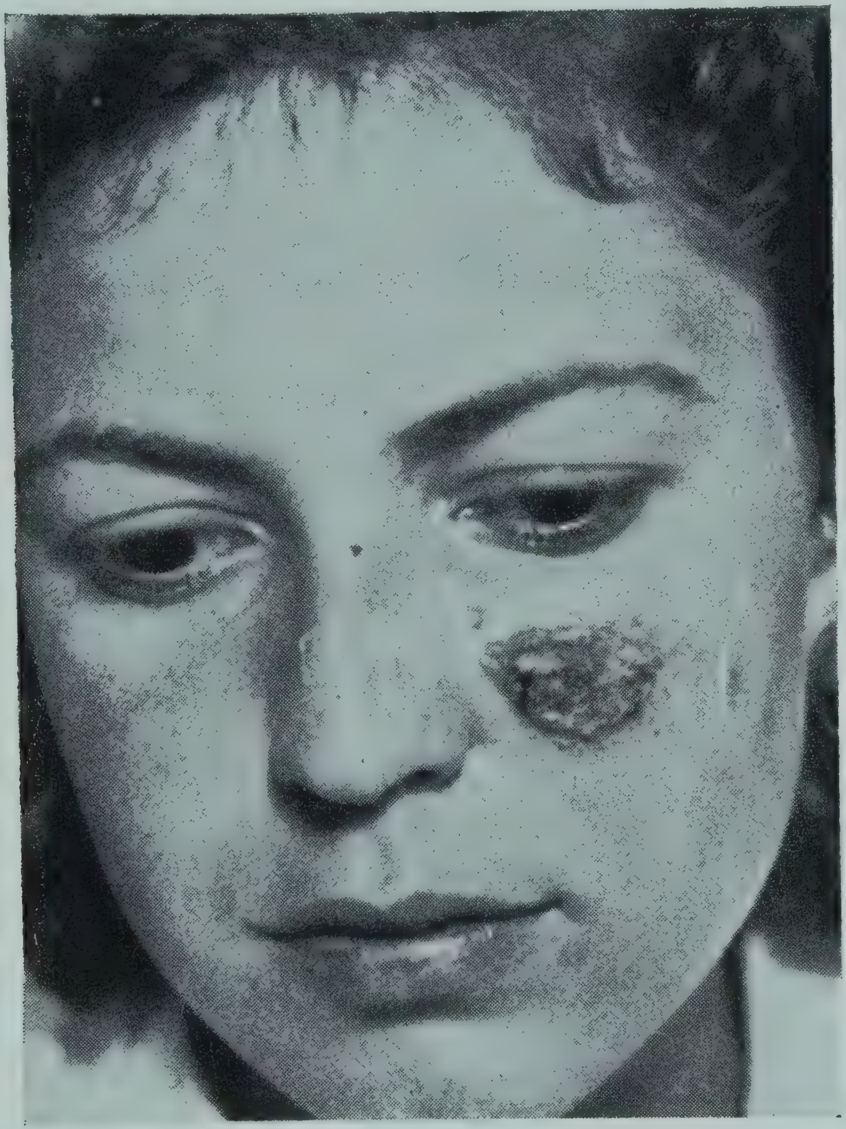

Fig. 7: Cutaneous leishmaniasis of three months' duration in a girl nineteen years of age. The lesion is ulcerative. Note the bubonulus-like nodules (lymphatic dissemination) above and to the nasal side of the lesion. (Courtesy of Dr. F. Sagher.)

HISTOPLASMOSIS

Histoplasmosis is a rare systemic disease involving the reticuloendothelial system, and it is caused by a species of fungus, Histoplasma capsulatum. Clinically, the disease suggests tuberculosis because of weight loss, night sweats, and fever. The enlarged spleen and liver help to differentiate it; but to establish the diagnosis, it is necssary to find the characteristic bodies.

Laboratory Examinations:

1. The Histoplasma capsulatum may be found in the monocytes of the peripheral blood or in bone-marrow smears from the sternum. The bodies stain blue or bluish-red with Wright's stain. They are oval, range from 2 to 4 microns in diameter, and are surrounded by a clear material that suggests a capsule.[21]
2. It may be cultivated on Sabouraud's medium, on blood agar, or by intraperitoneal inoculation of a guinea pig.

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CHAPTER 20

Heart and Blood Vessels

INFECTIONS OF THE HEART

Rheumatic Heart Disease:

Rheumatic heart disease occurs in over two-thirds of the cases of rheumatic fever. The actual cause of this disease is not known, but it is associated with infection with streptococci, which appear to enter through the upper respiratory tract. The heart and joint symptoms may be due to localized infection with the streptococcus or to allergy to this organism. The diagnosis is made by history and physical examination, but certain laboratory examinations yield helpful information.

Laboratory Examinations:

1. Blood cultures may contain streptococci, but in the majority of cases they are negative.
2. The sedimentation rate is increased when the disease is active. In fact, the sedimentation rate is the most reliable method of following the activity of the disease.
3. The leukocyte count is increased up to 15,000 or slightly more, and the polymorphonuclear neutrophils show an absolute increase.
4. Anemia is moderate when it is present. It is hypochromic and normocytic in type.

5. The urinary output is decreased (oliguria) during the acute phase of the disease. Albumin and erythrocytes are frequently found in routine examination.
6. In mitral stenosis, which is a sequel of the acute phase, pulmonary congestion takes place. As decompensation of the left auricle progresses, the sputum becomes rusty owing to the presence of heart-failure cells.

Acute Bacterial Endocarditis:

This is an acute infectious disease of the endocardium, particularly of the valves. The valves that are involved are those that have been previously damaged by rheumatic fever, arteriosclerosis, or congenital defects. Rarely does it involve the normal heart. Clinically, the patient has an acute infectious process with murmurs due to the development of vegetations on the injured valves. The endocarditis is likely to develop on damaged valves during infections, such as puerperal sepsis, pneumonia, acute tonsillitis, abscesses with bacteremia, and meningitis.

Laboratory Examinations:

1. A blood culture usually yields the causative organism when the disease is active. Hemolytic streptococci, pneumococci, Staphylococcus aureus, meningococci, and Escherichia coli are among the more common organisms found in this disease. Rarely, Streptococcus viridans, Eberthella typhosa, Hemophilus influenzae, Brucella melitensis, Pasteurella pestis, and Micrococcus tetragenus are isolated.
2. The sedimentation rate is increased.
3. The leukocyte count is increased even more than in rheumatic endocarditis. The polymorphonuclear neutrophils show an absolute increase.
4. A secondary anemia of the hypochromic normocytic type is noted.
5. If there is increased blood destruction or hepatitis from the causative organism, bilirubin is increased in the blood, the icterus index is increased, and the van den Bergh test is positive.
6. Albuminuria and hematuria are sometimes present if there is an acute infectious glomerulonephritis associated with the heart condition.

Subacute Bacterial Endocarditis:

This is a prolonged infectious disease of the endocardium, which usually involves those valves that have been previously damaged by rheumatic fever. Congenital valvular defects rarely constitute the background for the development of the disease. Streptococcus viridans is the causative organism in almost all of the cases.

Laboratory Examinations:

1. The Streptococcus viridans is usually found in blood culture, but it may require several examinations before the organism is found. Rarely, it is caused by Eberthella typhosa, Hemophilus influenzae, or gonococci.
2. The sedimentation rate is increased.
3. The leukocytes are moderately increased, and the increase is due to polymorphonuclear leukocytes.
4. Bacterial emboli or increased capillary permeability may result in hemorrhage from the organ involved.
5. When the bacteria involve the glomeruli, erythrocytes and albumin are found in the urine.
6. When the organism causes increased hemolysis of erythrocytes, bilirubin is increased in the blood, the icterus index is increased, and the van den Bergh test is positive.

Syphilitic Cardiovascular Disease:

The Treponema pallidum has a predilection for the ascending aorta and aortic valve. The vasa vasorum become obliterated, and the elastic tissue is destroyed. This results in aneurysm of the aorta. Narrowing of the ostia of the coronary arteries results in poor blood supply to the myocardium, but true syphilitic myocarditis is rare (0.3 per cent of cases).

Laboratory Examinations: Serologic tests for syphilis are positive in over 90 per cent of cases. A positive test merely indicates the presence of syphilis. Syphilitic aortitis and myocardial involvement must be diagnosed by clinical means.

Acute Pericarditis:

This is an acute infectious process involving the visceral and parietal layers of the pericardium. Acute fibrinous and purulent pericarditis may develop as a complication of certain diseases, such as rheumatic fever, septicemia, lobar pneumonia, bronchopneumonia, empyema, typhoid fever, tuberculosis, and infections with Hemophilus influenzae. Fibrinous pericarditis as a complication of uremia or a myocardial infarct is sterile.

Laboratory Examinations: The diagnosis of pericarditis is made by other than laboratory means, but certain bacteriologic findings help to confirm the nature of the infection:

1. When a pericardial tap is carried out, the bacteria found in culture depend upon the primary source of the infection. The common organisms are pneumococci, streptococci and staphylococci. Less common are Klebsiella pneumoniae, Hemophilus influenzae, Escherichia coli, Eberthella typhosa, and the Mycobacterium tuberculosis.

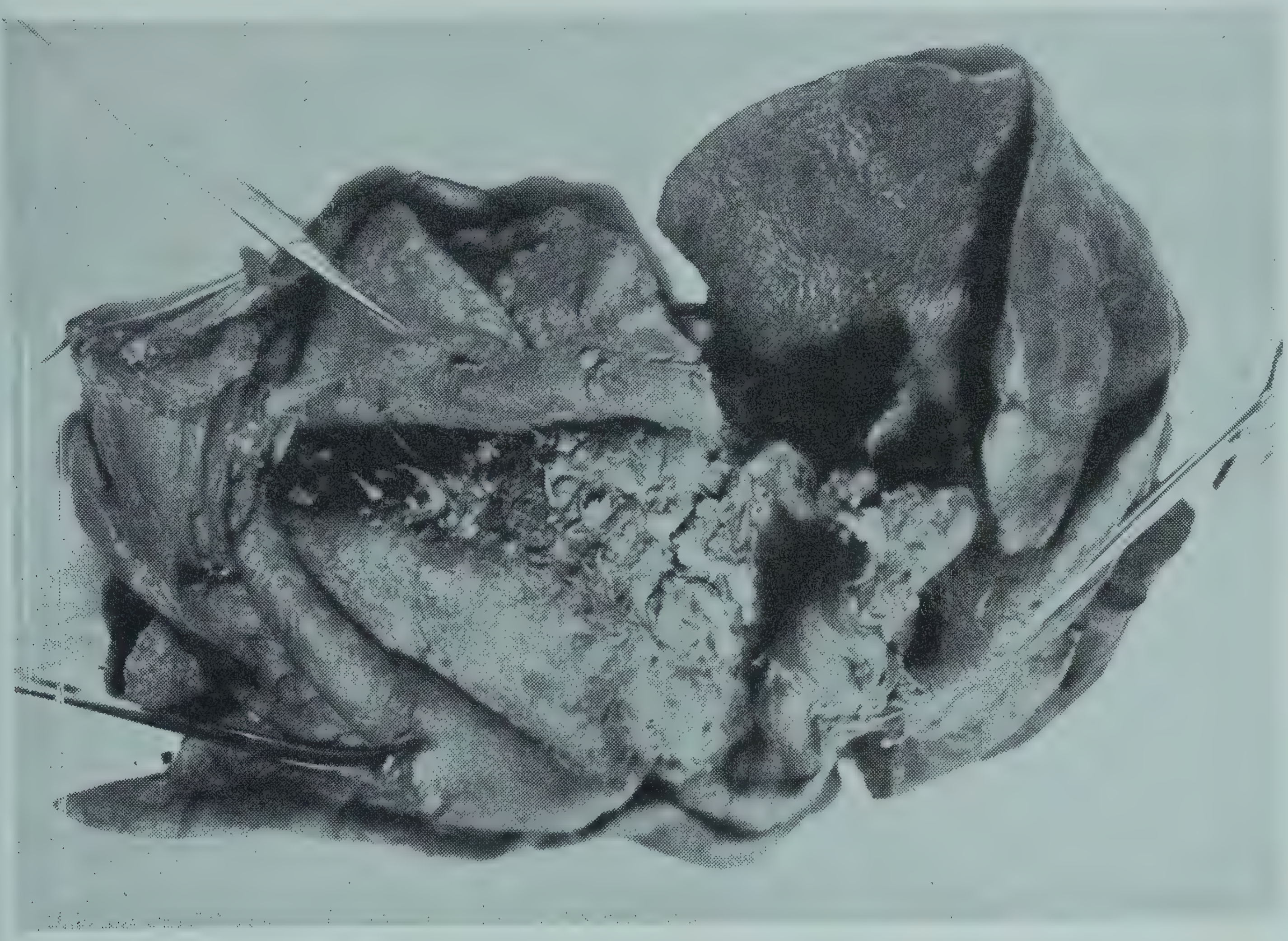

Fig. 1: Acute fibrinous pericarditis.

2. Cytological examination of pericardial fluid shows a marked preponderance of polymorphonuclear neutrophils. The total protein, specific gravity, and positive Rivalta test are consistent with the findings of an exudate.
3. The blood-leukocyte count is moderately increased, and there is an absolute increase in polymorphonuclear neutrophils.
4. The sedimentation rate is increased.
5. A secondary, hypochromic, normocytic anemia develops, particularly if the infection has run a prolonged course.

DISEASES CAUSING CARDIAC INSUFFICIENCY

Myocardial Insufficiency:

Congestive heart failure due to myocardial insufficiency is a sequel of chronic hypertension, myocardial infarction, myocardial degeneration as a result of poor blood supply in anteriosclerosis of coronary arteries, and stenosis of mitral and aortic valves. As a result, there is passive congestion of all organs, which causes certain changes in laboratory tests.

Laboratory Examinations:

1. In mitral stenosis, particularly, a secondary polycythemia may develop because of pulmonary congestion and anoxemia. However, in most cases, a hypochromic normocytic anemia is the usual finding.
2. Congestion of the kidneys is reflected by oliguria, increased specific gravity of urine, albuminuria, hematuria, and casts.
3. The phenolsulphonphthalein kidney-function test may be markedly reduced to as little as 10 per cent of the dye excreted.
4. Since kidney function is impaired, it is reflected in the blood by nitrogen retention. Thus, the values for nonprotein nitrogen, urea, uric acid and creatinine are increased.
5. Deficient carbon-dioxide elimination results in slight acidosis.
6. Slight increase in blood calcium is present because the increased carbon-dioxide tension allows the blood to keep more calcium in solution.
7. Bilirubin is slightly increased in the blood because of defective function of liver cells owing to inadequate oxygen supply. The icterus index is increased in proportion to the increase in bilirubin.

Congenital Heart Disease:

In congenital heart disease, the blood is incompletely oxygenated since some venous blood is shunted into the general circulation without passing through the lungs. This occurs in patent foramen ovale, patent ductus arteriosus, and interventricular defect.

Laboratory Examinations:

1. A polycythemia develops because of inadequate oxygenation of the blood. The red-cell count and hemoglobin are increased in proportion to the need for oxygen.
2. Oxygen saturation is markedly decreased.
3. Acidosis may develop to a slight degree.

4. Congestion of the kidneys is indicated by evidence of defective kidney function, that is oliguria, albuminuria, hematuria, casts, and nitrogen retention.

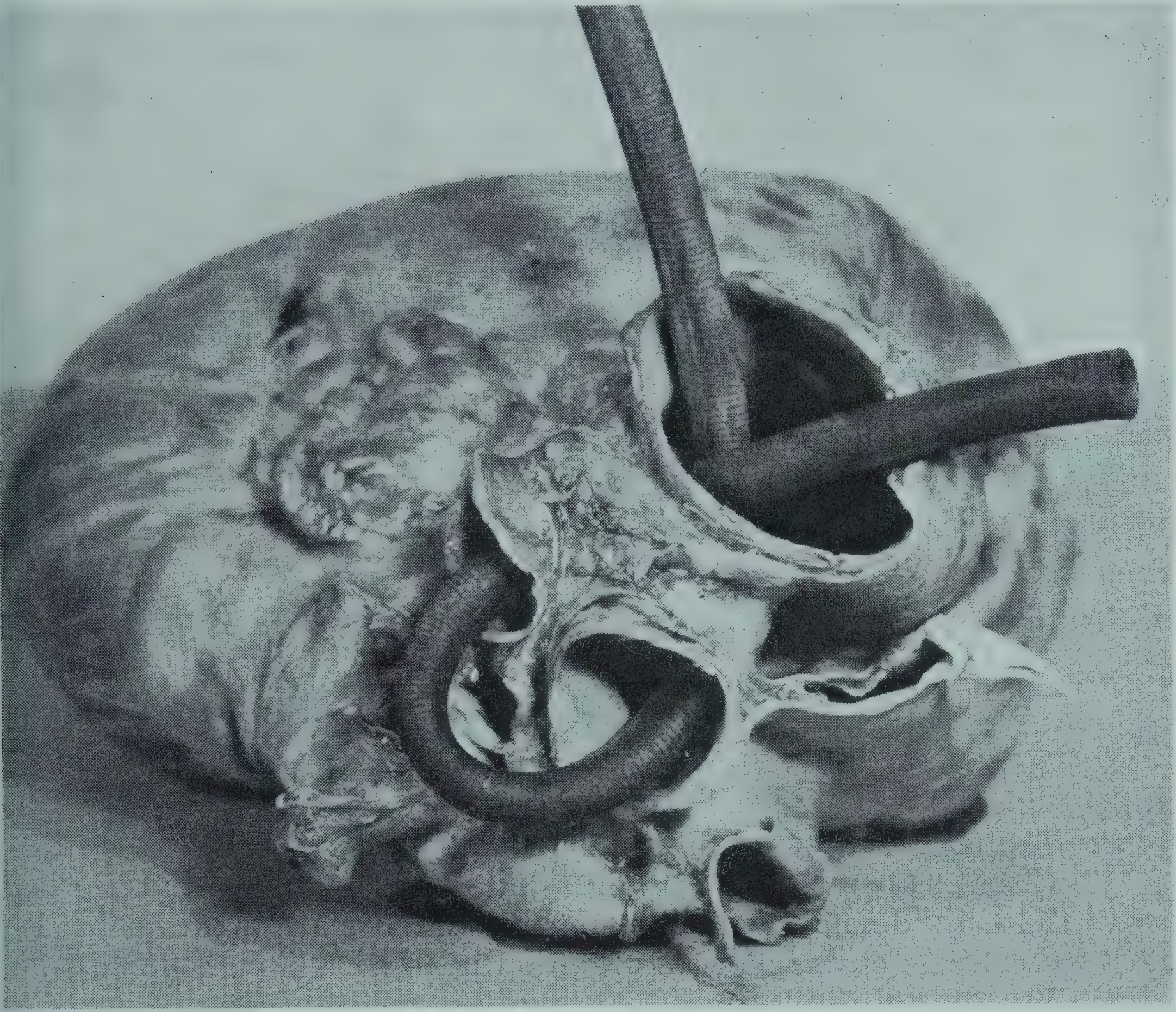

Fig. 2: Congenital anomaly of the heart: persistent truncus-arteriosus and septum-ventriculorum defect.

Coronary Artery Disease:

Coronary sclerosis, with or without thrombosis, results in myocardial insufficiency sooner or later. The diagnosis of either condition is not made by laboratory methods; but in sudden coronary thrombosis with infarction, certain laboratory tests are helpful.

Laboratory Examinations:

1. The leukocyte count is moderately increased: 12,000 to 16,000 per cubic millimeter. The polymorphonuclear leukocytes show an absolute increase.

2. The sedimentation rate is increased. It becomes normal with healing of the infarcted area.
3. Nonprotein nitrogen is slightly increased. This returns to normal with healing of the infarct.
4. Blood-cholesterol level is increased. In many persons with myocardial infarction, particularly those under forty-five years of age, cholesterol is increased and ranges from 250 to 350 mg. per 100 cc. or even higher. This increase is probably due to faulty cholesterol metabolism rather than to hypothyroidism per se.

DISEASES OF BLOOD VESSELS

Periarteritis Nodosa:

This is also called "polyarteritis nodosa." It is an acute inflammation with necrosis involving the walls of medium-sized and small arteries. The cause is not known, but it has been ascribed to viruses, rheumatic fever, streptococci, and allergy.

Laboratory Examinations:

1. Eosinophilia occurs in from 10 to 20 per cent of cases. It is usually moderate, but may reach 80 per cent.
2. The leukocytes are increased to from 15,000 to 30,000 with a distinct shift to the left.
3. The sedimentation rate is elevated.
4. When the blood supply to the kidney is interrupted with resulting localized infarction, albuminuria and hematuria develop. In extreme cases, nitrogen retention is noted.
5. Biopsy of a skin lesion or muscle serves to establish the diagnosis, provided the involved vessels are included in the section.

Thrombosis:

Heparin and dicumarol are now used extensively in the treatment of thrombosis. The coagulation time, prothrombin time, and prothrombin index are important in guiding the treatment with these drugs.

Treatment with Heparin: Heparin is a mucoitin sulfuric acid which has a high molecular weight and a strong negative charge. The exact mechanism whereby heparin prevents coagulation of blood is not clearly understood. There is, however, a quantitative antagonism between thromboplastin and heparin. Blood that is rendered incoagulable by heparin will clot if sufficient thromboplastin is added to it. Heparin also opposes the action of thrombin. The presence of salts and some protein factor of the plasma seems necessary for heparin to exert its characteristic action.

The occurrence of hemorrhage following heparin treatment is very rarely encountered unless it is used too soon after an operation. For this reason, heparin is used by some clinicians without checking the coagulation time in patients with normal renal function. Loewe and associates[1] believe that the coagulation time should be determined daily while heparin is used. They recommend a modification of the Lee-White method[2] for the determination of the coagulation time. The method is as follows:

1. Place four, chemically clean, dry, 75 by 10 mm. (3 by ⅖ inch) test tubes in a rack.
2. With a sterile dry syringe and needle, withdraw a little less than 2 cc. of venous blood from the patient.
3. Distribute a little more than 0.35 cc. of blood into each test tube, and allow the tubes to stand until the test is completed.
4. The test is timed by stop watch from the moment the vein is negotiated to the time clotting is noted in the tubes. Normal values range from nine to fifteen minutes. If clotting occurs at different times in the four tubes, average the three closest figures.

In a patient under treatment with heparin, a coagulation time of forty-five to sixty minutes is satisfactory to obtain effective clinical results. In fact, Loewe and co-workers[1] state that coagulation times of more than one hour are unnecessary and indicate waste of heparin.

The effect of heparin is almost immediate, while that of dicumarol takes about forty-eight hours. Some clinicians start heparin and dicumarol together and stop heparin at the end of forty-eight hours so that no time is lost in instituting effective treatment.

Treatment with Dicumarol: Schofield's[3] discovery of the part played by spoiled sweet clover in hemorrhagic disease of cattle was followed by the isolation and synthesis of the active principle. The active principle is dicoumarin, which is sold under the trade name of "dicumarol." Dicumarol produces its effect by preventing the formation of prothrombin in the liver. About forty-eight hours are required for it to produce its effect.

In order to know the patient's normal values, a prothrombin time and prothrombin index are determined before dicumarol is started. As stated in Chapter I, the normal prothrombin time is ten to twenty seconds, and the normal prothrombin index is 70 to 100 per cent. It is advisable to do a daily prothrombin index until the daily dosage of dicumarol is established. After this, a determination every other day is sufficient. The patient should receive enough of the drug to maintain the prothrombin index at 20 to 60 per cent, which is effective in preventing thrombosis and is safe from danger of hemorrhage. However, Davidson and MacDonald[4] recommend 10 to 30 per cent of normal.

TABLE 1: SUMMARY OF LABORATORY EXAMINATIONS IN ANTICOAGULANT THERAPY

Anti-coagulant	*Test*	*Normal Values*	*Optimal Values during Treatment*
Heparin.....	Coagulation time (modification of Lee-White method)	9 to 15 minutes	45 to 60 minutes
Dicumarol...	Prothrombin time (Quick method)	12 to 20 seconds	35 to 80 seconds
Dicumarol...	Prothrombin index	70 to 100 per cent	20 to 60 per cent
Dicumarol...	Per cent of prothrombin activity (Quick)	70 to 100 per cent	15 to 25 per cent

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CHAPTER 21

The Respiratory System

VITAL CAPACITY

In preparing patients for chest surgery, it is important to know the vital capacity. The total amount af air that can be exhaled after a maximum inspiration is called the "vital capacity." For healthy adult males, it is about 4000 cc., but figures vary for different persons and for each sex. The test should be repeated in order to secure an accurate figure. The computation is based upon height, weight, and sex.

The vital capacity is reduced by the following:

1. Chronic pulmonary infections, such as tuberculosis and bronchiectasis
2. Compression of one or both lungs by pleuritic or pericardial effusion, pneumothorax, or tumors
3. Mechanical interference with movement of the diaphragm or thoracic cage
4. Pulmonary edema and chronic passive congestion of the lungs, secondary to a decompensated heart

INFECTIONS OF THROAT AND LARYNX

Vincent's Angina:

This is a local infection that involves the gingival borders, tonsils, uvula, nasopharynx, or nose. It is characterized by ulceration and a brownish-gray membrane.

Laboratory Examination:

1. Examination of the stained smear reveals large numbers of Borrelia vincentii mixed with Fusiformis dentium. Elimination of these organisms cures the disease. It is important to differentiate this disease from diphtheria and from a syphilitic lesion that may simulate it. Both Vincent's spirochetes and Treponema pallidum may be demonstrated by darkfield examination in syphilitic lesions.

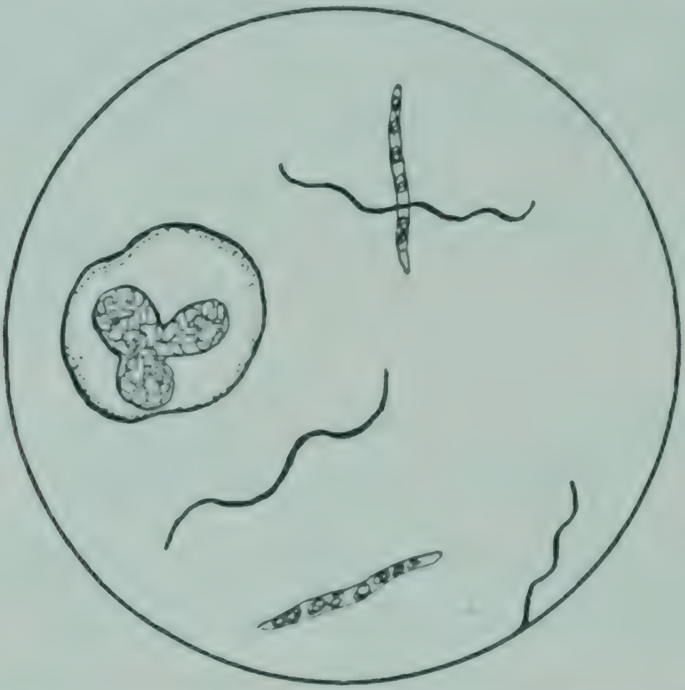

Fig. 1: Vincent's organisms (Borrelia vincentii and Fusiformis dentium).

2. In a severe case with systemic reaction, there is frequently a moderate leukocytosis with an increase in polymorphonuclear neutrophils.

Diphtheria:

This is a local infection caused by the Corynebacterium diphtheriae, but it has serious systemic manifestations because of the potent exotoxin formed by the organism. The throat, larynx, and trachea or nose may be involved.

Laboratory Examinations:

1. Because of the urgency for the administration of antitoxin, immediate smear and culture should be taken. Diphtheroid bacilli and Corynebacterium pseudodiphthericum may be confused with the Corynebacterium diphtheriae; but if there is any doubt, it is safer to administer antitoxin.
2. Determination of virulence by guinea-pig inoculation is sometimes advisable, particularly if there is any doubt about the identity of the organism.

3. A leukocytosis of a moderate degree is usually present, and there is an absolute increase in polymorphonuclear neutrophils. In the occasional case of severe diphtheria, there may be a leukopenia.
4. The diphtheria toxin produces a transient glomerular nephritis. With this, the urine will show albumin, red cells, and casts.
5. The Schick test is of little value in diagnosing the active case, because diphtheria may occur in a mild form in a Schick-negative person.

Scarlet Fever:

This is a systemic infection characterized by a reddish papular rash and caused by a betahemolytic streptococcus (Streptococcus scarlatinae). Complications and mortality rate are lower since the advent of the sulfonamides and penicillin.

Laboratory Examinations:

1. Streptococcus scarlatinae is readily cultured from the throat. However, the final diagnosis must be made clinically because betahemolytic streptococci may be isolated in cases of epidemic sore throat. The condition must be differentiated bacteriologically from other causes of throat infection, such as the Corynebacterium diphtheriae, staphylococci, and Vincent's organisms.
2. The Schultz-Charlton test is positive in scarlet fever and serves to differentiate this disease from others with similar rashes. It is performed by an intracutaneous injection of 0.1 cc. of scarlet-fever antitoxin into an area where the rash is of deepest color. After twenty-four hours, a positive test is characterized by blanching around the red spot where the injection was made.
3. There is usually a leukocytosis of 12,000 to 30,000 or more per cubic millimeter, and the neutrophils reach 90 per cent in some cases. A slight eosinophilia is present at the end of the first week and during the third to fifth weeks.
4. A moderate secondary anemia usually develops after the first week of the untreated disease.
5. Albumin and casts are present in the urine during the febrile stage. In some cases, acute glomerular nephritis develops with oliguria and a large amount of urinary albumin, red cells, and casts.

Pertussis (Whooping Cough):

This is a local infectious disease of the respiratory tract, which is believed to be caused by Hemophilus pertussis. Although the diagnosis can usually be made clinically, it is sometimes helpful to identify the organism.

Laboratory Examinations:

1. Hemophilus pertussis can be cultured on appropriate media, which is seeded by droplets while coughing (cough plate). It can also be cultured from material obtained from the nasopharynx on a swab.[1] The highest proportion of positive results is obtained during the first two weeks of the disease.
2. The leukocyte count is increased and ranges from 15,000 to 30,000 per cubic millimeter. The lymphocytes are increased and range up to 60 per cent or more. These findings are frequently helpful when found in conjunction with a characteristic type of cough. However, it must be remembered that children under five years of age have a normal lymphocyte range of 35 to 50 per cent.
3. Other diagnostic measures have been tried and have not been found satisfactory for general clinical use. They may be summarized as follows:
   a. The complement-fixation test is positive in only about 50 per cent of cases according to Kolmer[2] and Paton.[3]
   b. The agglutination titer of Hemophilus pertussis is increased in whooping cough, but the titer is not built up rapidly enough to make the test of clinical value.
   c. Intradermal skin tests with the vaccine or toxin have been tried by various authors.[3-6] But again, the patient does not become sensitive early enough to make the test of practical value.

Tuberculosis of the Larynx:

Tuberculosis of the larynx is usually secondary to pulmonary tuberculosis.

Laboratory Examinations: Bacteriological examination and microscopic examination of the stained tissues obtained by biopsy are helpful procedures in establishing a diagnosis. Thus, other processes that may occur in the larynx, such as papilloma, carcinoma, mycotic infections, and leprosy, may be excluded.

Syphilis of the Larynx:

Gumma of the larynx is the most common form of laryngeal syphilis. It is difficult to establish this diagnosis, but a positive serological test and laryngoscopic examination are helpful.

Laboratory Examinations:

1. A positive serological test for syphilis should be established.
2. A biopsy of the lesion is helpful in excluding the diseases mentioned under Tuberculosis of the Larynx.

INFECTIONS OF LUNGS

Bacterial Pneumonia

Pneumococcal Pneumonia:

This type of pneumonia is caused by the pneumococcus of which there are forty-one different types. Since the advent of the sulfonamides and antibiotics, the determination of the types of pneumococcus is not so important as it was when serum therapy was used. Some still advise the typing of pneumococci in case a given organism is resistant to the therapeutic agent used. Examination of the sputum and blood culture are particularly important in this type of pneumonia.

Other Types of Pneumonia:

Lobar pneumonia and bronchopneumonia are caused by a variety of organisms other than the pneumococcus. These organisms include Streptococcus hemolyticus, Staphylococcus aureus, Klebsiella pneumoniae, Hemophilus influenzae, Hemophilus pertussis, Pseudomonas aeruginosa, Bacillus anthracis, Pasteurella pestis, Brucella melitensis, Eberthella typhosa, Escherichia coli, Malleomyces mallei, Pasteurella tularensis, meningococci, and Treponema pallidum.

Laboratory Examinations:

1. Although many cases are treated without knowing the causative organism, it is desirable to establish the etiological agent when possible. To this end bacteriological examination of the sputum should be carried out.

   The sputum should be collected in a sterile container and sent to the laboratory covered. Material may also be obtained from children and others who do not expectorate by obtaining material from the posterior nasopharynx on swabs.

   A direct smear of the sputum stained by the Gram technic is helpful in determining the predominating organisms. The culture usually confirms the presence of the predominating organism in the smear.

Typing of the pneumococcus and Hemophilus influenzae is desirable, but it is not always ordered by the clinician. Moist preparations are helpful in determining the presence of mycotic infection.

2. Blood cultures should be carried out in all cases of pneumonia. The culture media must be fortified with *para*-aminobenzoic acid and penicillinase so that the sulfonamides or penicillin used in treatment will be neutralized and will not inhibit growth in vitro. A positive blood culture makes the prognosis more serious.
3. There is usually a leukocytosis ranging from 18,000 to 30,000 or more per cubic millimeter with an absolute increase in polymorphonuclear leukocytes. Children tend to have a higher leukocyte count than adults. Some persons who are particularly toxic may have a leukopenia, in which case the prognosis is guarded. A continued leukocytosis with polymorphonuclear leukocytes predominating suggests a complication, such as abscess or empyema. When a leukocytosis shows a predominance of lymphocytes, it suggests delayed resolution.
4. The sedimentation rate is prolonged markedly until the crisis.
5. Platelets are frequently reduced, and the coagulation time is concurrently prolonged.
6. Jaundice is a complication of some penumonias owing to toxic hepatitis. When this occurs, the icterus index is increased, and the direct van den Bergh test is positive.
7. A toxic nephrosis develops and with it albumin and casts in the urine. Albumin, casts, and erythrocytes in the urine indicate glomerulonephritis, which is a complication in some cases.
8. Nitrogen retention is present with nephrosis and nephritis. There is a moderate increase in the nonprotein nitrogen, blood-urea nitrogen, and uric acid.
9. There is frequently an increase in plasma protein, primarily because of an increase in globulin.
10. The blood chloride is frequently reduced, although this is variable. The blood calcium is increased because calcium is kept in solution by the variations in carbon-dioxide tension.

Empyema:

Before the use of sulfonamides and antibiotics, empyema was a more common complication of pneumonia than at present. It may be caused by any of the organisms that cause bronchopneumonia or lobar pneumonia. It may also be the result of a penetrating wound of the chest, and the infection is caused by the organisms' being carried into the pleural cavity. Extension from mediastinitis, pericarditis, and subphrenic abscess is also a cause of empyema. Tuberculous empyema is the most common type seen in those who have pulmonary tuberculosis.

Laboratory Examinations:

1. Bacteriological examination of the empyema fluid by smear and culture is necessary so that the causative organism may be found. If the mycobacterium tuberculosis is not found on direct smear when tuberculous empyema is suspected, the material should be cultured on appropriate media or inoculated into a guinea pig.

   In addition to aerobic and anaerobic pyogenic organisms, appropriate culture media may reveal Pasteurella tularensis, Hemophilus influenzae, Candida albicans, Actinomyces bovis, Nocardiae, Coccidioides immitis, and Sporotrichum shenckii. A stained smear or darkfield examination may reveal Vincent's organisms.
2. In pyogenic empyema, there is a leukocytosis due to an absolute increase in polymorphonuclear neutrophils. In pure tuberculous empyema, the leukocytosis is only slight.
3. Albumin and casts are present in the urine when a toxic nephrosis is present.

Lung Abscess:

Lung abscess may be a complication of the following:

1. Lobar pneumonia or bronchopneumonia
2. Septic infarct
3. Foreign body in a bronchus
4. Bronchiectasis
5. Obstructing tumor in a bronchus
6. Extension or rupture of a subphrenic abscess
7. Traumatic perforation of a lung
8. Pyogenic infection of a parasitic, tuberculous, or mycotic lesion

Laboratory Examinations:

1. Bacteriological examination of sputum or material obtained by bronchoscopic examination may reveal the infecting organism or organisms. Both aerobic and anaerobic cultures should be carried out. The organisms that may be found in a lung abscess are various types of streptococci and staphylococci, pneumococci, Klebsiella pneumoniae, Hemophilus influenzae, Neisseria catar-

rhalis, Gaffkya tetragena, and Vincent's organisms (Borrelia vincentii and Fusiformis dentium).

2. Blood culture should be done because of the possibility of septicemia.
3. The leukocyte count is markedly increased due to neutrophilic leukocytosis.
4. The sedimentation rate is increased.

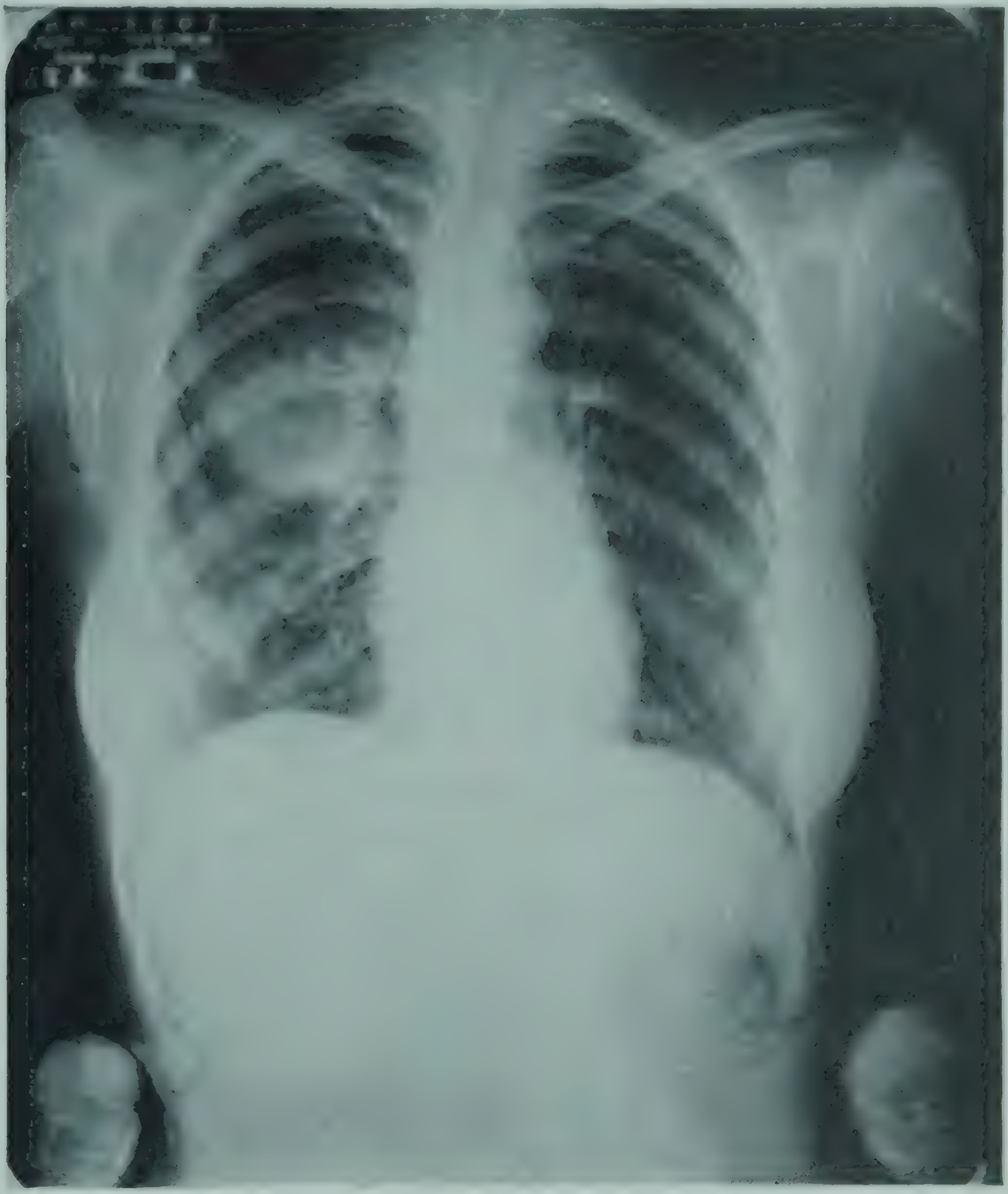

Fig. 2: X-ray film of abscess of right lung.

Bronchopulmonary Spirochetosis:

This disease is apparently caused by the same spirochetes and fusiform bacilli that infect the mouth. They are carried to the lungs by aspiration.

Laboratory Examinations: Examination of the stained smear of the sputum reveals the spirochetes and fusiform bacilli. Darkfield examination shows the characteristic large, loosely wound spirochetes.

Pulmonary Tuberculosis:

Because of the danger of spreading the Mycobacterium tuberculosis, all cases of suspected tuberculosis should be thoroughly investigated by every means available. Although the history, physical examination, and x-ray plate of the chest may suggest pulmonary tuberculosis, the absolute proof of the diagnosis rests with the laboratory.

Laboratory Examinations:

1. Examination of the plain or concentrated sputums for mycobacterium tuberculosis is of primary importance in establishing a diagnosis. The sputum may be concentrated by the use of antiformin, chlorox, or other methods. Although in practically all cases, acid-fast bacilli in the sputum are Mycobacteria tuberculosis, the technician is justified in reporting only "acid-fast bacilli" in the stained smear. The number of organisms present may be reported according to the Gaffky scale as shown in Chapter 13 (p. 261).

   Sputums that are negative for acid-fast bacilli in concentrated specimens may be cultured or inoculated into a guinea pig. Since preparation of a sputum for culture does not always kill the nontuberculous, acid-fast bacilli, it seems preferable to inoculate a guinea pig.
2. If sputums are negative, the fasting gastric contents should be inoculated into a guinea pig. This is advisable because some persons swallow the sputum and with it Mycobacteria tuberculosis.
3. With the increasing use of streptomycin in treating certain types of pulmonary and extrapulmonary tuberculosis, certain strains are becoming resistant to the drug. Ideally, the sensitivity of the organism to streptomycin should be determined before starting treatment. The following technic, modified from that used by Youmans,[7] has been used successfully in determining the sensitivity of Mycobacteria tuberculosis to streptomycin:
   - *a.* Prepare concentration of streptomycin equal to 100,000 micrograms per cubic centimeter in Dubos media.

     One gram streptomycin plus 10 cc. Dubos media equals 10 cc. solution (each cubic centimeter contains 100,000 micrograms). This is the stock solution and may be kept in the refrigerator for ten days. (By using phosphate buffer diluent, it may be kept for four months in refrigerator.)

b. Make working solutions as follows:

Solution 1. (1000 µg./ml.): Take 1 cc. of stock, and make up to 100 cc. with Dubos (or 0.5 cc. stock to 50 cc. Dubos)

Solution 2. (100 µg./ml.): Take 1 cc. of 1:1000, and make up to 10 cc. with Dubos.

Solution 3. (50 µg/ml.): Take 1.25 cc. of 1:1000 solution and 23.75 ml. Dubos media.

Solution 4. (5 µg./ml.): Take 2.5 cc. solution of 1:50 µg./ml. and 22.5 Dubos media.

TABLE 1: SENSITIVITY PROTOCOL

Tube No.	*Cc. Streptomycin (µg./ml.)*	*Cc. Dubos Media*	*Final Concentration (µg./ml.)*
1.	5 cc. of 1000	0	1000
2.	5 cc. of 100	0	100
3.	0.5 cc. 100	4.5	10
4.	1.00 cc. of 5	4.0	1
5.	(Control) 0	5.0	0

c. Wash entire slant of patient's Mycobacterium-tuberculosis culture into Dubos media, and grow five to seven days.

d. Inoculate all five tubes with 0.1 cc. of patient's organisms growing in Dubos media. Read at five days, seven days, and up to fourteen days.

e. Interpretations of results: Sensitivity of the organism is determined by the smallest amount of streptomycin that will inhibit growth.

A strain of Mycobacterium tuberculosis is "resistant" if growth occurs in 10 or more µg./ml., and "sensitive" if there is inhibition by 10 or less µg./ml. of streptomycin.

4. The tuberculin skin test is of value in adults only when it is negative. The positive test does not distinguish between active and inactive disease. It is of some value in young children and infants because a positive test usually means active disease.
5. The leukocyte count usually ranges from 8,000 to 12,000 per cubic millimeter, but it may be subnormal. Lymphocytes are frequently increased. A mild, hypochromic, normocytic anemia may be present.
6. When the disease is active, the sedimentation rate is increased. Since the sedimentation rate parallels the activity of the disease,

periodic tests are helpful in determining favorable or unfavorable progress.

7. Extensive pulmonary tuberculosis causes the oxygen saturation of arterial blood to fall below 95 per cent, which is the lower limit of normal.
8. The vital capacity is reduced in proportion to the amount of lung tissue involved.
9. Plasma protein may be either normal or slightly increased. The increase is due to a rise in the globulin level. Fibrinogen is frequently increased from a normal of 250 mg. to from 400 to 1000 mg. per 100 cc. Cholesterol may be decreased in severe cases.
10. In febrile cases, the urine may contain albumin.

VIRAL PNEUMONIA

Primary Atypical Pneumonia:

At least six viral agents other than influenza virus, psittacosis virus, and lymphocytic-choriomeningitis virus have been isolated by various investigators in the search for the cause of primary atypical pneumonia. At present, it is believed that the cause is a virus, although it may be complicated by a bacterial infection.

The patient first experiences chills followed by a cough, which may be productive of blood-streaked sputum. There is pain in the anterior chest, and later dyspnea and cyanosis may develop.

Laboratory Examinations:

1. The cold-agglutinin titer is increased up to 1:224 or higher. An increasing titer with a characteristic chest x-ray is strong evidence in favor of viral pneumonia in a patient with a pneumonic process.
2. The leukocyte count is moderately increased (12,000 to 20,000 per cubic millimeter), but it may be even higher. The polymorphonuclear neutrophils are increased and may constitute as high as 90 per cent of the total leukocytes.
3. The sputum usually contains streptococci and staphylococci.
4. The blood culture is negative.

Psittacosis:

This is an atypical type of pneumonia with fever and prostration. It occurs as an infectious disease of birds, particularly parrots, and is characterized by nasal discharge, diarrhea, weakness, and moping. Man is infected by inhaling the virus from the discharges from infected parrots.

Laboratory Examinations:

1. Injection of blood or sputum into the peritoneal cavity of a mouse will produce a characteristic infection, which will kill the mouse in a week. Examination of the liver and spleen shows focal necrosis, and intracellular bodies may be demonstrated.
2. The complement-fixation test is positive.

FUNGOUS INFECTIONS OF LUNGS

Coccidioidomycosis:

This is primarily a fungous infection of the lungs, which is caused by Coccidioides immitis and is limited at present to the region of the San Joaquin Valley, California. The disease is contracted by inhaling the chlamydo-spores, which develop in the lungs and cause the primary pulmonary infection. It may be limited to the lungs, it may spread generally throughout the body, or it may involve the lungs and skin. The spread of the organism throughout the body apparently takes place by way of the bloodstream.

Laboratory Examinations:

1. Microscopic examination of the sputum may reveal the organism. If this is negative in a suspected case, culture and guinea-pig inoculation should be carried out. The pulmonary form of this disease is difficult to differentiate from pulmonary tuberculosis until the etiological agent is identified.
2. The intradermal skin test with coccidioidin is highly specific.

Aspergillosis:

This is a rare form of mycotic pulmonary infection, which is caused usually by Aspergillus niger or Aspergillus fumigatus.

Laboratory Examinations: Microscopic and cultural examinations of the sputum are used to identify the presence and type of Aspergillus. Since this fungus is ubiquitous, its presence in the sputum does not necessarily mean that it is causing the pulmonary infection. In fact, it may be found in the saliva and sputum of normal persons.

To prove that this fungus is the cause of a lung lesion, the patient should wash his mouth thoroughly and then cough up sputum from the bronchi directly into a sterile container. The presence of large numbers of organisms in such a specimen, found on repeated examinations, makes the Aspergillus the probable cause of the pulmonary lesion.

Blastomycosis:

This disease is caused by Blastomyces dematitidis, which enters the body through the injured skin. Although it begins as a skin lesion, it may spread and involve any organ, particularly the lungs.

Laboratory Examinations: The sputum should be examined microscopically for the organism, but it is not satisfactory. Examination of pus from a skin lesion is more likely to demonstrate the yeast.

Actinomycosis:

This infection is caused by Actinomyces bovis, which may be found in the normal mouth. It is the most common type of systemic fungous infection, but only about 15 per cent of the cases involve the lungs.

Laboratory Examinations:

1. Microscopic examination of the sputum for identification of the characteristic ray fungus is the first step in proving the pulmonary form of the disease. The same care should be observed in collecting the sputum as described under Aspergillosis.
2. Anaerobic culture and guinea-pig inoculation should be carried out if the direct examination is negative.

Parasitic Infections of Lungs

Paragonimiasis:

This disease is caused by the lung fluke, Paragonimus westermani. Man is infected by eating raw crabs or crayfish, which contain the metacercariae. In the duodenum, they come out of the cysts, pass through the intestinal wall, and traverse the abdominal cavity and diaphragm to the lungs where they mature in a capsule formed by the host.

Laboratory Examinations: The brownish flecks in the sputum are examined for ova of Paragonimus westermani.

Pulmonary Amebiasis:

Amebic abscess may develop in the lungs as a complication of liver abscess, which ruptures through the diaphragm. It is caused by Endamoeba histolytica, which occurs primarily as an intestinal infection (amebic dysentery).

Laboratory Examinations: Microscopic examination of the sputum may reveal the parasite. Of course, the stools should be examined to establish the presence of the parasite in the body.

Although the larval stage of Ascaris lumbricoides, Strongyloides stercoralis, and the hookworms involve the lungs, examination of the sputum does not usually aid in the diagnosis because the larvae are rarely expectorated.

ALLERGIC CONDITIONS

Rhinitis:

Rhinitis is a manifestation of allergy. It is usually caused by one of several agents, which may be inhaled and to which the patient is sensitive.

Laboratory Examinations:

1. Skin tests are of primary importance to find out the particular substance to which the person is sensitive.
2. Examination of a smear of nasal secretion shows large numbers of eosinophils when it is an allergic rhinitis. If polymorphonuclear neutrôphils predominate, the rhinitis is bacterial in origin, or pus is draining from a sinus.

Hay Fever and Asthma:

Hay fever is a seasonal disease caused by sensitization to the pollens of certain grasses and weeds. Asthma is a symptom complex, which may or may not be caused by sensitivity to various substances.

Laboratory Examinations:

1. Skin tests should be carried out to determine the particular agent or agents to which the patient is sensitive. See Skin Tests, Chapter 16, p. 303.
2. In truly allergic cases, there is usually an eosinophilia of 5 per cent or more.

3. In chronic asthma, the sedimentation rate may be increased, particularly if there is a secondary infection.

MISCELLANEOUS

Pneumoconiosis:

Pneumoconiosis is a chronic pulmonary disease, which is the result of inhaling an irritating substance over a long period of time. The dusts that cause the disease are silicon, hydrated magnesium silicate (asbestos), carbon, iron, and calcium. Modern devices to protect workers make pneumoconiosis less common than formerly.

Laboratory Examinations: Asbestosis bodies may be found in the sputum. Chemical examination of the sputum may be of help in identifying the agent.

Bagasse Disease (Bagassosis):[8] This is an industrial disease resulting from exposure to the dust of bagasse, or sugar cane after it has been crushed and the juice has been extracted. This by-product of the sugar industry is finding increasing use in the manufacture of acoustical and thermal insulating materials and in the production of refractory brick.

The disease has been reported in the United States in Louisiana and Missouri, in Cuba, and in England where these insulation products are made.

Bagasse dust consists of pith particles with a small quantity of rind fibers, assorted bacteria, and fungous spores. There is some salica in the ash, but the amount is thought to be insignificant.

The disease is an acute febrile illness, which comes on after about two months' exposure. The patient has extreme dyspnea, marked weakness, and a persistent cough with a scanty mucoid sputum. X-ray examination reveals an extensive punctate infiltration of the lungs, which may coalesce into pneumonic areas. This picture is not characteristic of this disease, but it is similar to other pneumoconioses. The pulmonary changes are reversible; and in those who have recovered, there appear to be no residual effects.

LABORATORY EXAMINATIONS:

1. Examination of the sputum is not striking, but it may contain some of the materials in the bagasse dust, such as pith, fibers, and fungous spores.

2. The blood shows a leukocytosis with a shift to the left.
3. An eosinophilia is present.

Tumors:

The diagnosis of accessible tumors of the respiratory system is best made by microscopic examination after biopsy or complete removal. A biopsy can be taken from laryngeal and visible intrabronchial tumors. If a tumor is not visible through the bronchoscope, bronchial washings should be taken for cystological study after special staining, such as the Papanicolaou method.[9] Study of the bronchial washings gives a much higher proportion of positive findings than similar study of the sputum.

Biopsy of a metastatic lymph node in the lower neck sometimes confirms the diagnosis of broncogenic carcinoma in the presence of x-ray evidence.

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CHAPTER 22

Gastrointestinal Tract and Pancreas

In addition to laboratory and x-ray diagnostic procedures for the intestinal tract, present practice includes the use of esophagoscopy, gastroscopy, proctoscopy, and peritoneoscopy. The actual visualization of lesions plus biopsies when indicated add much to the clinician's diagnostic armamentarium in diseases of the gastrointestinal tract.

MOUTH AND PHARYNX

Bacterial infections of the mouth and pharynx are discussed in Chapter 13 (p. 267), and mycotic infections of this region are discussed in Chapter 15 (p. 298).

Tumors of the mouth are readily accessible for biopsy and microscopic diagnosis.

ESOPHAGUS

Infection:

Infection of the esophagus is rare and is usually associated with mediastinitis. Extension of moniliasis from the mouth into the esophagus occurs on rare occasions.

Tumors:

Biopsy of esophageal tumors through an esophagoscope is carried out in order to diagnose the nature of the process.

Varices:

Esophageal varices develop secondary to cirrhosis of the liver. Rupture of one of these varices frequently produces enough hemorrhage to cause hematemesis. In any event, bleeding from a ruptured varix is a source of occult blood in the stool.

STOMACH AND DUODENUM

Acute Gastritis:

The stomach reacts to irritants by increasing the gastric secretion to dilute the agent and to protect the mucosa. The irritants may be chemical, mechanical, bacterial, or thermal.

Laboratory Examinations:

1. In case of a poison that may be taken intentionally or accidentally, the vomitus should be subjected to chemical examination.
2. The presence of mucus is a significant finding.
3. In a gastric analysis, the free-hydrochloric-acid level is variable. It may be normal, or it may be neutralized by regurgitated, alkaline, duodenal contents.
4. A stained smear of the gastric contents after a test meal of alcohol may contain leukocytes, particularly in bacterial and phlegmonous gastritis.

Peptic Ulcer:

This disease includes ulcers of the stomach, duodenum, and rare ulcers of the esophagus and jejunum. Although the specific cause of the process is not known, certain predisposing factors are recognized. They are septic tonsils, alveolar abscesses, burns, septicemia, uremia, erysipelas, and nervous tension. The primary lesion is an area of necrosis of the mucosa, which may be due to embolus, hemorrhage, or vascular spasm. The necrotic tissue is subject to the digestive action of the enzymes of the gastric juice, and ulcer results. Peptic ulcer is usually diagnosed by history and x-ray examination. Laboratory findings are confirmatory and serve to complete the picture.

Laboratory Examinations:

1. In gastric ulcer, the free hydrochloric acid and total acidity may be increased, normal, or reduced after a test meal; but after administration of histamine, both are usually increased above

normal. About half of the cases have hyperchlorhydria after a test meal only. In duodenal ulcer, the free hydrochloric acid and total acidity are more consistently increased above normal after the test meal as well as after histamine.

2. The volume of the fasting gastric contents is increased from about 50 cc. to 100 cc. or more.
3. The alkali reserve is sometimes increased, particularly in the presence of excessive secretion of hydrochloric acid by the stomach.

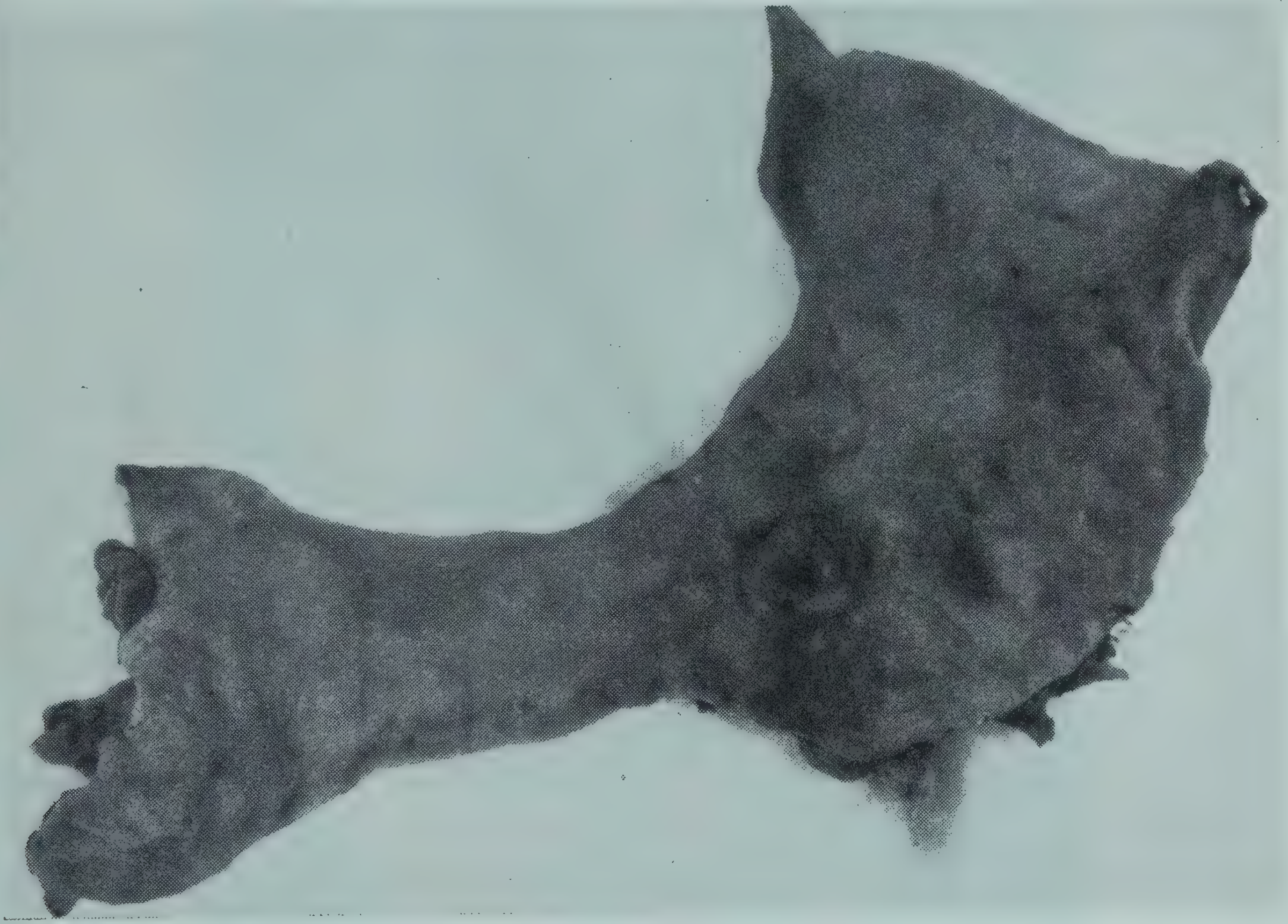


Fig. 1: Gastric ulcer associated with increased hydrochloric acid.

4. Hemorrhage from chronic peptic ulcer results in a hypochromic anemia. The erythrocyte count ranges from 4,000,000 to 2,500,000 per cubic millimeter or lower depending on the amount and duration of the hemorrhage. Such hemorrhage results in a positive test for occult blood in the gastric contents and stool. In fact, the stool may have a tarry appearance from the large amount of blood that has been subjected to the digestive process in the intestines.
5. Hypochloremia may develop in cases receiving alkali therapy.
6. Nitrogen retention develops following severe hemorrhage if there is defective kidney function. This is apparently owing to the digestion of a large amount of blood.

Carcinoma of Stomach:

The diagnosis of gastric carcinoma is usually made by x-ray examination or gastroscopy. Examination of the gastric contents yields supplementary information, which may be confirmatory.

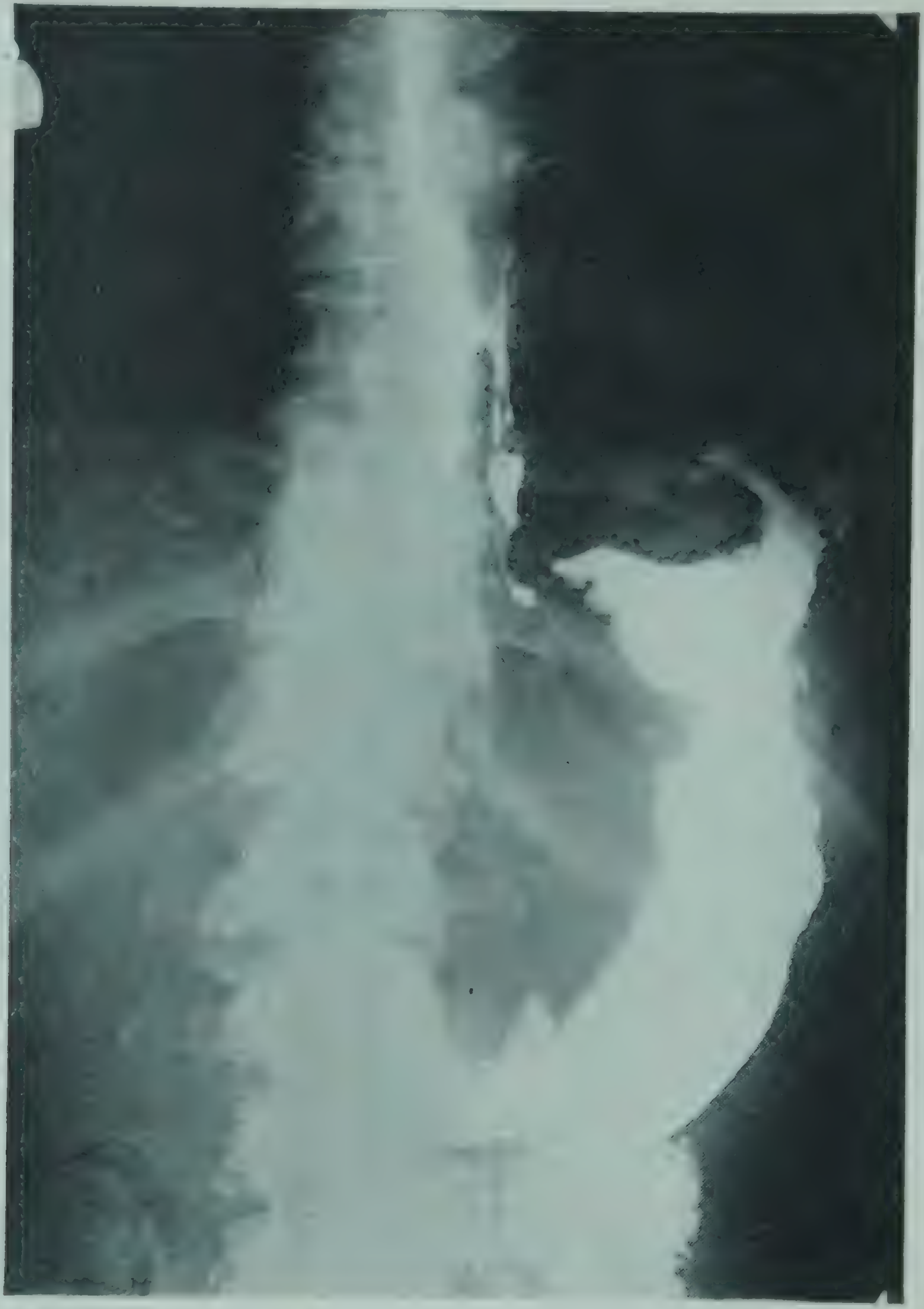


Fig. 2: X-ray film demonstrating carcinoma of fundus.

Laboratory evidence does not differentiate carcinoma from other malignant tumors of the stomach.

Laboratory Examinations:

1. Achlorhydria is the characteristic sign in carcinoma of the stomach. Absence of hydrochloric acid and of total acidity is noted in about two thirds of the cases. Hypochlorhydria and hypoacidity are found in the remainder.

2. The volume of gastric secretion is decreased. However, in cases of obstruction, the volume retained in the stomach is increased.
3. In obstruction with stagnation of gastric contents, Lactobacillus acidophilus and Lactobacillus bulgaricus are usually present.
4. Both the gastric contents and feces contain occult blood in most cases of carcinoma, particularly if there is any necrosis.
5. A normocytic hypochromic anemia usually develops. Myelophthisic anemia develops if the carcinoma metastasizes to the bone marrow.
6. Examination of a stained smear from the gastric contents shows tumor cells in an appreciable percentage of cases. They are characterized by variation in size and shape and deeper staining quality of the nuclei.

INTESTINES

Tuberculosis:

Tuberculosis of the stomach, duodenum, and jejunum is rare, but infection of the ileum and colon is fairly common in advanced pulmonary disease. The infection is spread from the lungs, probably by the bloodstream or by swallowing the organisms. Direct infection by Mycobacterium tuberculosis bovis is rare today.

Laboratory Examinations: Examination of the concentrated preparation of feces by direct smear and guinea-pig inoculation may reveal Mycobacteria tuberculosis. It must be recognized that these organisms may have been swallowed. However, if the Mycobacterium tuberculosis is the only pathogenic agent found in a diarrheal stool containing mucus and blood from a patient with advanced pulmonary tuberculosis, it strongly suggests intestinal tuberculosis.

Appendicitis:

The diagnosis of acute appendicitis is an ever-present problem for the clinician. In addition to the history and physical examination, the leukocyte count and differential count constitute part of the routine work-up.

Laboratory Examinations:

1. The leukocyte count varies from a normal of 8000 to 20,000 per cubic millimeter in adults. In children, the range is higher (12,000 to 30,000 per cubic millimeter).

2. The Schilling index is helpful if other foci of infection have been ruled out. It is of value in persons with normal or moderately increased leukocyte counts as pointed out by Goodale and Manning.[1] Yaguda[2] feels that the Schilling index is helpful in diagnosing appendiceal infection, whether acute or chronic. However, others[3,4] have little or no confidence in the Schilling index as an aid in diagnosing appendicitis.
3. The sedimentation rate is normal in early appendicitis. This fact is sometimes used to differentiate acute appendicitis from acute salpingitis for in the latter disease, the sedimentation time is shorter, and the rate is increased from the beginning. However, later in appendicitis, the sedimentation rate is increased, and it is then of no value in differentiating the disease from salpingitis.
3. The urine should be examined routinely to rule out urinary-tract infection as a cause of pain.

Bacterial Infections:

In addition to the normal bacterial flora of the intestines, pathogenic organisms are sometimes ingested and cause dysentery or systemic disease. These bacteria are the Shigellae (dysentery bacilli), Salmonellae (including typhoid and paratyphoid bacilli), and paracolon, cholera, and anthrax bacilli.

For discussion of the laboratory findings in these infections, see Chapter 27 (pp. 516 and 524).

Diarrhea of Newborn:

The cause of epidemic diarrhea of the newborn has not been determined. The variety of findings in cultures of the nose, throat, and stools suggests that we are dealing with an organism or virus that present methods have failed to isolate. The organisms most frequently isolated have been Escherichia coli, Escherichia coli mutabile, Shigella dysenteriae (Sonne), Klebsiella pneumoniae, paracolon bacilli, Proteus morgani, atypical organisms of the dysentery group, Pseudomonas aeruginosa, Streptococcus viridans, enterococci, and yeasts.

Laboratory Examinations:

1. The carbon-dioxide combining power is reduced, sometimes as low as 5 or 10 volumes per cent.

2. Bacteriologic examination of the feces should be carried out in all epidemics.
3. Nonprotein nitrogen and total protein are increased because of dehydration.
4. Blood chloride is increased if there is no vomiting.
5. Leukocytes are moderately increased.
6. Red cells and hemoglobin are increased because of concentration of the blood.
7. Albumin and casts are found in the urine.

Gastrointestinal Toxicosis in Children:

The severe vomiting and diarrhea are associated with infections elsewhere in the body or with epidemic diarrhea. The result is dehydration with hemoconcentration and acidosis.

Laboratory Examinations:

1. The carbon-dioxide combining power is markedly reduced, sometimes as low as 5 or 10 volumes per cent.
2. Nonprotein nitrogen is increased because of dehydration.
3. Blood chloride is decreased because of loss of hydrochloric acid in vomiting.
4. Red cells and hemoglobin are increased because of hemoconcentration.
5. Oliguria is usually observed, and albumin and casts may be present in the urine.

Chronic Ulcerative Colitis:

The cause of chronic ulcerative colitis is not known. It may be the final stage of some as yet unrecognized bacterial or virus infection. The various bacteria that have been isolated as causative agents do not appear to hold up against accumulating evidence.[5,6]

Laboratory Examinations:

1. Stools are characterized by the presence of mucus, blood, leukocytes, and epithelial cells.
2. Bacteriological, parasitological, and chemical examinations of the stools should be carried out to eliminate other disease entities, such as bacillary dysentery, amebiasis, and chronic metallic poisoning.
3. The sedimentation rate is increased.
4. Secondary anemia is usually present.

Intestinal Obstruction:

There are many causes of intestinal obstruction. When the lumen of the bowel is completely obstructed as a result of an adhesion band, kinking, volvulus, pressure by a tumor, intussusception, strangulation by a hernial ring, paralytic ileus, masses of Ascaris lumbricoides or Taenia saginata, mesenteric thrombosis, or a gallstone, symptoms of intense severity result. The loss of fluid and electrolytes in the vomitus and the drainage of fluid into the distended bowel lead to a fall in blood chloride, alkalosis, and dehydration.

Laboratory Examinations: Although the diagnosis of intestinal obstruction may be made by physical examination and x-ray examination of the abdomen, laboratory examinations reveal the status of body fluids and electrolytes:

1. The blood chloride is reduced because of the loss of hydrochloric acid in the vomitus and the acumulation of secretions in the intestine just above the point of obstruction.
2. The alkali reserve is increased early in the disease up to 80 volumes per cent. This is owing to an increase of sodium and potassium in the blood in the form of bicarbonate.

   In the presence of starvation, acidosis may develop with acetone in the urine and a reduced alkali reserve. Further, if for some reason pancreatic secretions and bile are not available, acidosis may develop because of reduction in plasma base.
3. The plasma protein is increased, and the albumin-globulin ratio usually remains unchanged. Part of the hyperproteinemia is due to an increase in fibrinogen.
4. Kidney function is impaired because of dehydration, and azotemia supervenes. The nonprotein nitrogen may be increased to 250 mg. per cent; and urea nitrogen, uric acid, and creatinine are proportionately increased.
5. With dehydration, there is hemoconcentration, decreased blood volume and increased viscosity.
6. The sedimentation rate is normal or decreased.
7. Leukopenia occurs in the face of abdominal distention; but when peritonitis develops, a leukocytosis is seen.
8. The urine usually contains indican. While the dehydration persists, the urine contains albumin and casts, and the twenty-four-hour volume is decreased oliguria).
9. Inorganic phosphates and blood cholesterol are increased.

Steatorrhea:

This disease occurs as idiopathic steatorrhea in infancy and childhood (celiac disease) and as symptomatic steatorrhea in both children and adults. Celiac disease is a chronic nutritional disturbance characterized by a lack of absorption of fatty acids from the intestine. Symptomatic steatorrhea is characterized by an excess of neutral fat in the feces, which indicates a deficiency in the splitting of fats.

Classification: Steatorrhea may be classified as follows:

1. Celiac disease (idiopathic steatorrhea)
2. Symptomatic steatorrhea
    - *a.* Due to pancreatic deficiency
        1. Congenital pancreatic insufficiency
        2. Pancreatitis
        3. Carcinoma
    - *b.* Obstructive jaundice
    - *c.* Gastroenteritis
    - *d.* Mechanical obstruction of lacteals

Celiac Disease: This disease is characterized by (1) large, pale, foul stools containing an excess of fatty acid and soap; (2) abnormal gastrointestinal motility; (3) enlarged abdomen and weak atrophied muscles; (4) dehydration crises; (5) symptoms of vitamin and mineral deficiency; and (6) chronic course with impairment of growth.

LABORATORY EXAMINATIONS:

1. Total fat is increased in the feces with most of the increase due to fatty acids and soap. Neutral fat and fatty acids may be detected microscopically.
2. The stool is characteristically bulky and pale.
3. The carbon-dioxide combining power may fall to 20 volumes per cent.
4. Azotemia develops in the presence of oliguria, and the nonprotein nitrogen may reach 100 mg. per 100 cc. When the patient is hydrated, the nonprotein nitrogen returns to normal.
5. The serum proteins may fall to 4 gm. per 100 cc.
6. A low total cholesterol (100 to 140 mg. per 100 cc.) and low total blood lipids are observed until definite improvement takes place.
7. Gastric enzymes are present in normal amounts.[7] According to Andersen,[8] pancreatic enzymes are also normal except during periods of dehydration when they are temporarily reduced.

8. In most cases, there is a hypochromic normocytic anemia. The erythrocytes vary from 3,000,000 to 5,000,000 per cubic millimeter. Occasionally, there is a hyperchromic anemia associated with a leukopenia and thrombocytopenia.

Pancreatic Deficiency. LABORATORY EXAMINATIONS:

1. Total fat is markedly increased. Most of it is due to neutral fat. Fatty acids and soap are essentially normal.
2. Nitrogen of the feces is increased.
3. Many undigested muscle fibers are present.
4. Blood calcium is reduced.
5. Hyperglycemia is present, and glucose tolerance is decreased.
6. Blood diastase is usually increased.
7. Glycosuria is observed fairly frequently.
8. A hypochromic normocytic anemia is usual.

Obstructive Jaundice. LABORATORY EXAMINATIONS:

1. The feces is greasy and light-colored.
2. Total fat is markedly increased, mostly owing to fatty acids and soap. Neutral fat is normal.

Gastroenteritis: Steatorrhea develops because of increased intestinal motility. Undigested and unabsorbed foods including fats are found in the feces.

LABORATORY EXAMINATIONS: Total fat may be increased to 50 per cent above normal with an increase in neutral fat, fatty acids, and soap.

Obstruction of Routes of Digestion and Absorption: Fat absorption from the intestine may be disturbed by the following: (1) a short circuit of intestine (gastrocolic fistula); (2) lesions that alter the peristalsis; or (3) obstruction of the lacteals by lymphoma or, as Veghelyi[9] reported, by Giardia infestation.

LABORATORY EXAMINATIONS: Total fat is increased in the feces, most of it owing to fatty acids and soap.

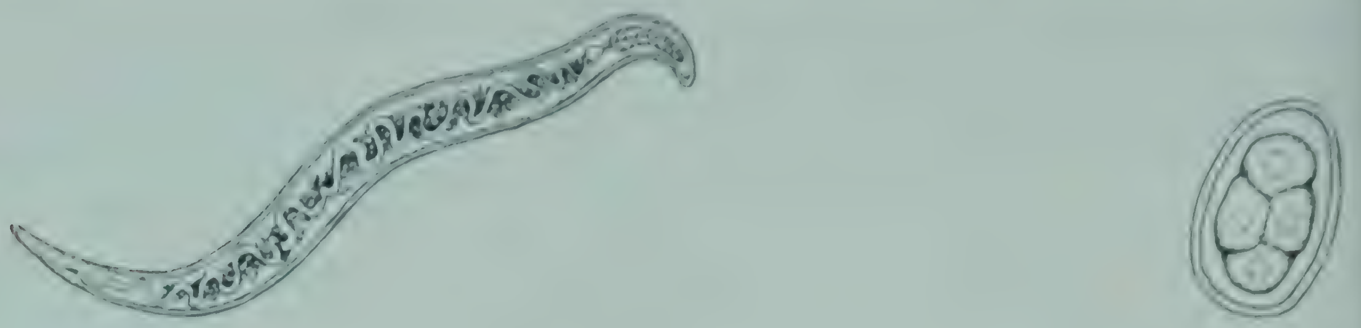
Intestinal Parasites:

Intestinal parasites are discussed in Chapter 8 (p. 197), but they are summarized here together with other pertinent laboratory findings.

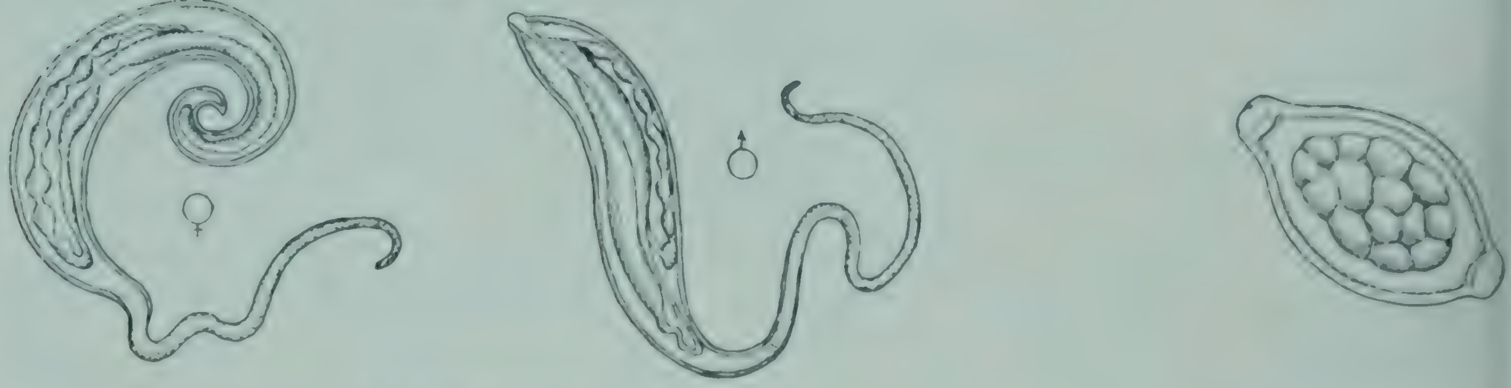
Protozoa:

Endamoeba histolytica:	1. Vegetative forms are found in the fresh dysentery stools. Cysts are found in carriers or convalescent cases. 2. The Craig[5] complement-fixation test is positive. Craig reserves this test for the diagnosis of carriers and suspected cases in which the parasites cannot be found. 3. Endamoeba histolytica may be cultured on special media, but it is not so satisfactory as direct examination of the fresh stool.
Endamoeba coli, Endolimax nana, Iodamoeba buetschlii, and Dientamoeba fragilis:	1. These amebas are not considered pathogenic. They are found in the feces of a small number of normal persons. All of these amebas have both cyst and vegetative forms except Dientamoeba fragilis, for which no cyst form is recorded.
Balantidium coli:	1. This is a rare form of infestation, particularly in the temperate zone. 2. Examination of the feces reveals a large, double-walled cyst with a kidney-shaped nucleus. The motile ciliated form may be found in the feces during the acute disease.
Intestinal flagellates (Giardia intestinalis, Chilomastix mesnili, Trichomonas hominis, Retortamonas intestinalis, and Enteromonas hominis):	1. These parasites are usually harmless to man. They have been found most frequently in the feces of diarrheal patients because these stools are more frequently examined. Some believe that Giardia intestinalis may cause diarrhea in children. 2. The diagnosis of all but Trichomonas hominis is made by finding the characteristic cyst or, more rarely, the motile form. No cyst form has as yet been found for Trichomonas hominis.

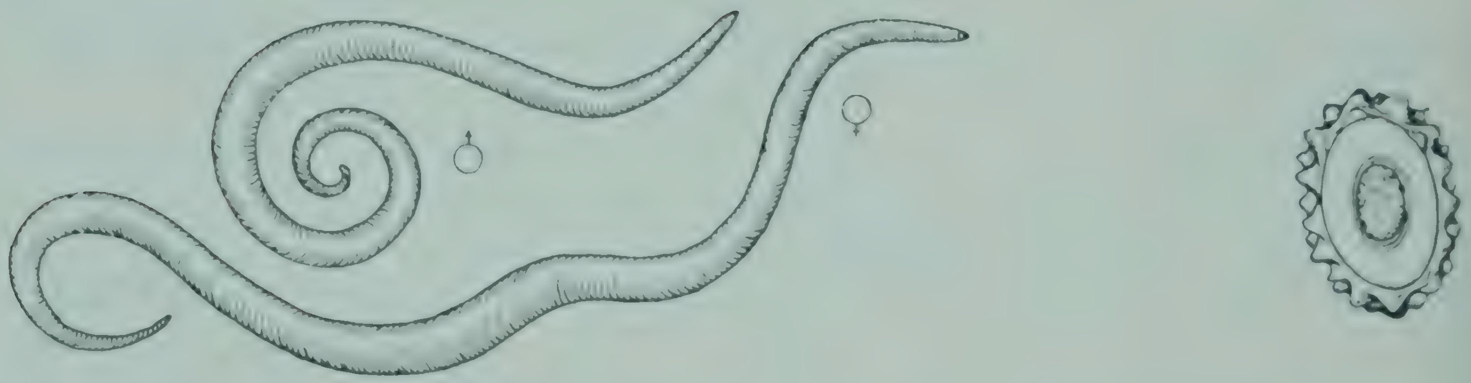
Helminths: The important helminths parasitic for man include the roundworms (Nematehelminthes) and flat worms (Platyhelminthes). The important class of Nematehelminthes is the Nema-



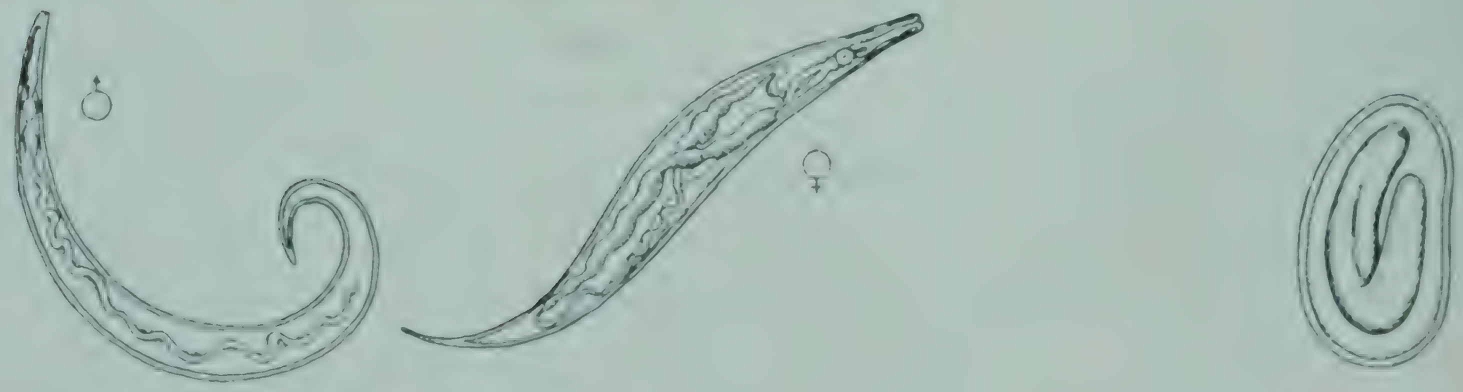
Necator americanus



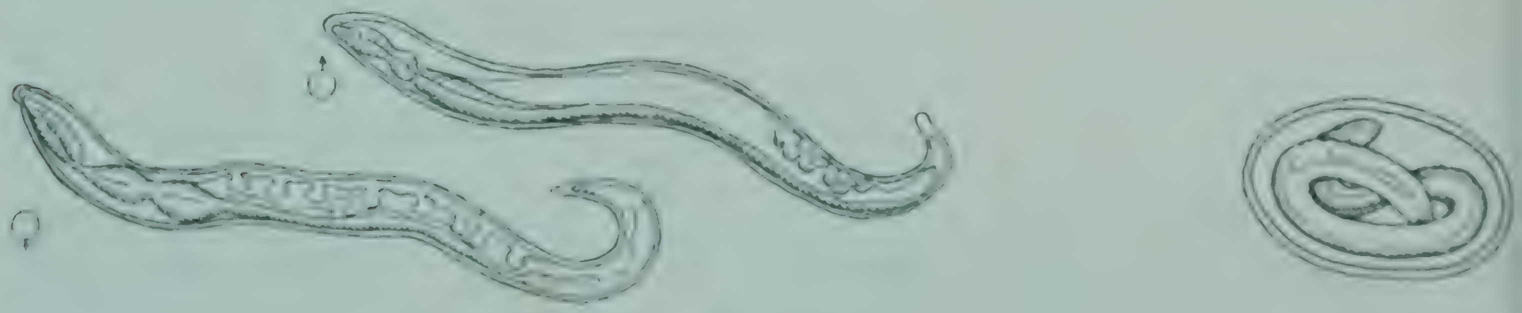
Trichuris trichiura



Ascaris lumbricoides



Oxyuris vermicularis



Strongyloides stercoralis

Fig. 3: Nematoda, adult worms and ova.

toda. Two classes of Platyhelminthes are found in man, the Trematoda (flukes) and Cestoda (tapeworms).

NEMATODA

Ascaris lumbricoides:

1. The diagnosis is made by finding the characteristic mammillated ova or adult worms in the feces. The adult worm is sometimes vomited up by children.

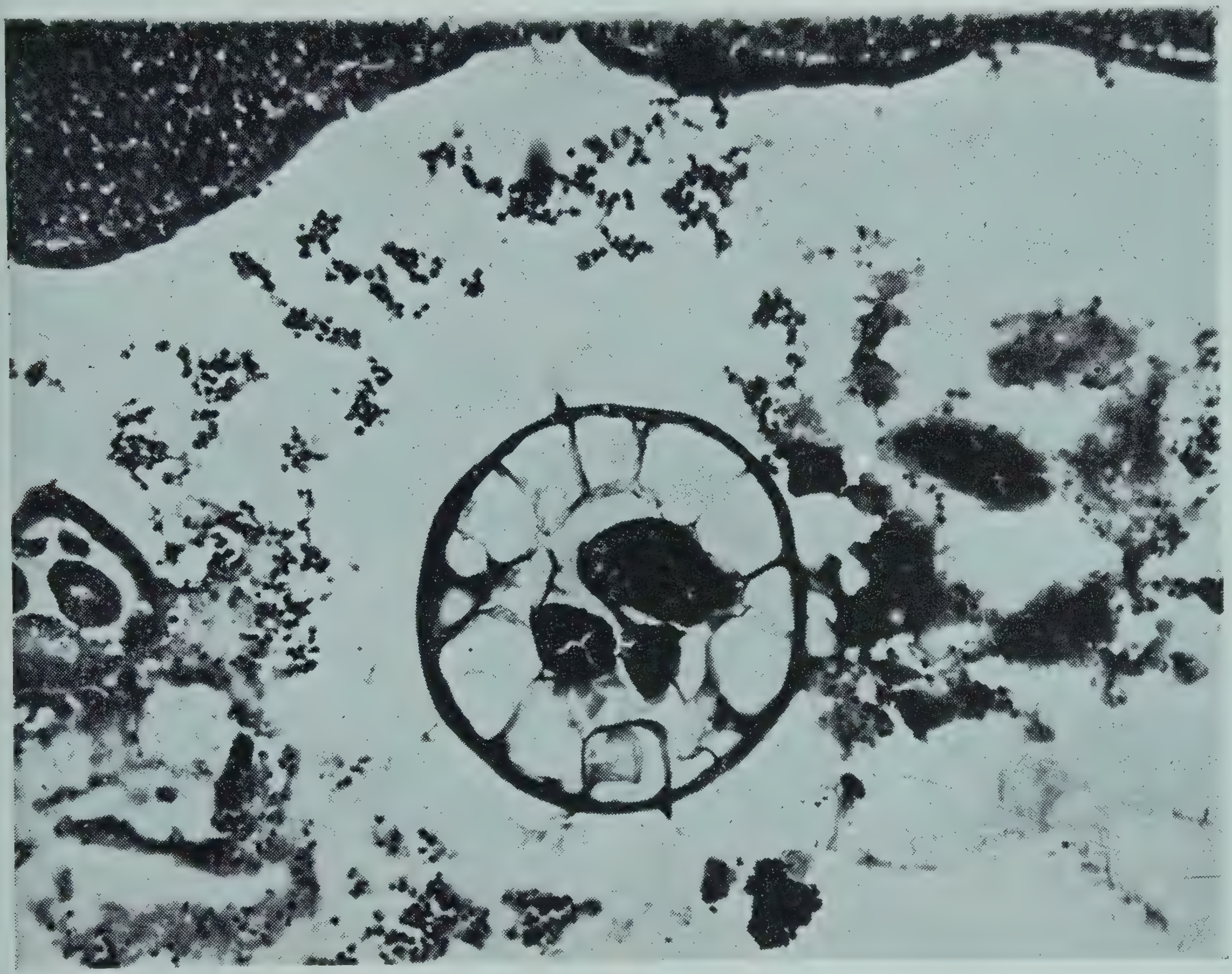


Fig. 4: Cross section of Enterobius vermicularis in lumen of appendix.

2. Eosinophilia is found when the patient is allergic to the emanations.
3. Secondary anemia is seen in massive infestations.

Trichuris trichiuria:

1. The oval ova are found in the feces. They are characterized by a clear plug in each end.
2. A slight eosinophilia may be present.

Enterobius vermicularis:

1. The planoconvex ova or the adult worms are found in material from the anus or vagina. The ova are rarely found in the feces. The ova may be found around the anus by rolling the sticky surface of scotch tape over the anus. The tape is placed around the forefinger with the sticky side out; and after securing the material, it is stuck to a slide and examined. The National Institute of Health swab may also be used.

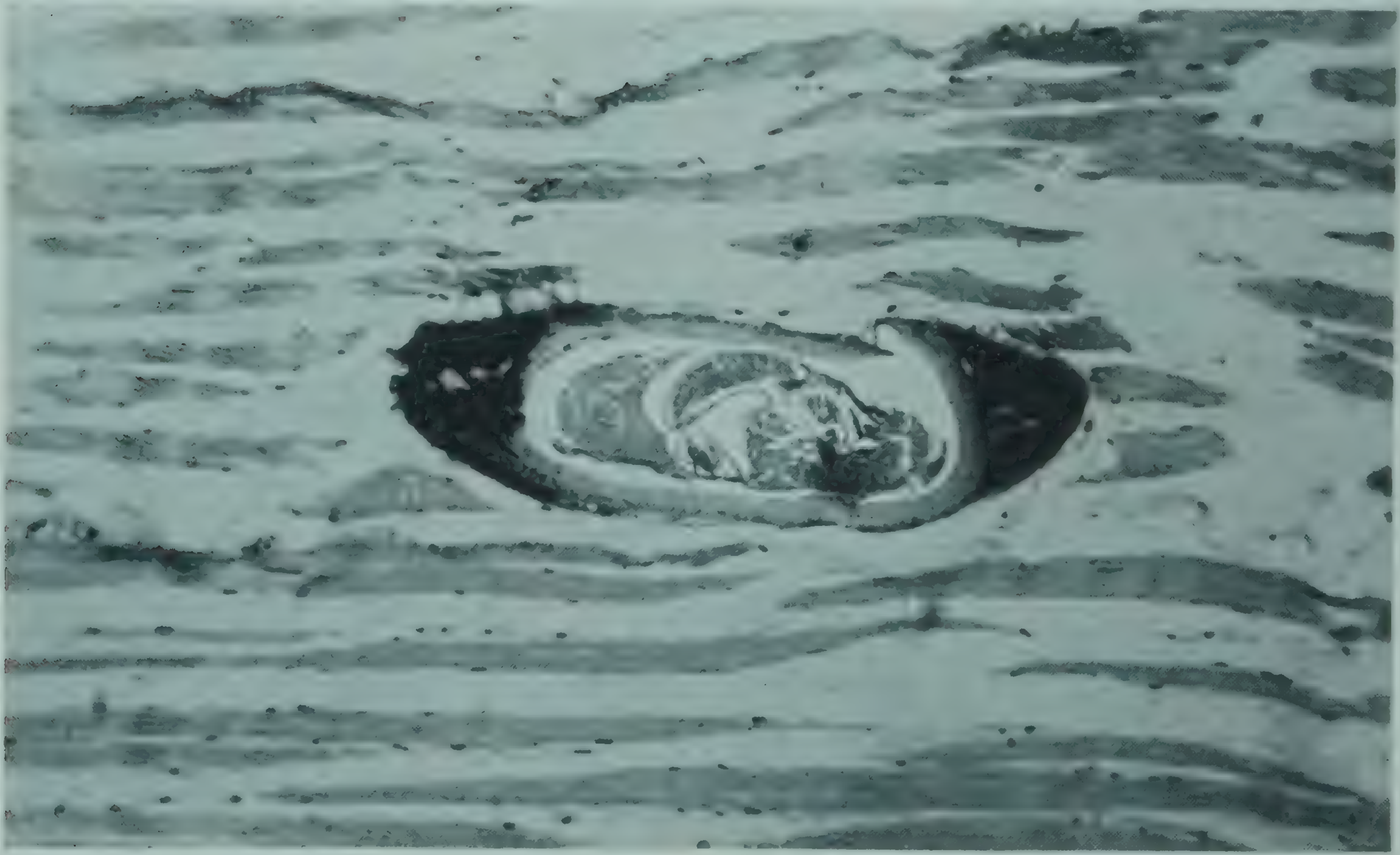


Fig. 5: Trichinella spiralis in muscle.

2. Adult worms may be found in the lumens of appendices, particularly in children.
3. Eosinophilia occurs occasionally.

Hookworms

Necator americanus and ancylostoma duodenale:

1. Oval segmented ova are found in the feces. These ova are colorless and have two to eight cells or segments.
2. Eosinophilia is moderate.
3. In chronic untreated cases with massive infestation, a hypochromic macrocytic anemia is present.

Strongyloides stercoralis:

1. Embryonated ova or rhabditic larvae are found in the feces.

2. Eosinophilia is marked early in the disease.
3. Leukocytosis is present in the early stage.
4. In chronic infection, there is a slight eosinophilia and an increase in lymphocytes.

Trichinella spiralis:

1. Although this parasite is ingested in pork, the diagnosis is not made by examining the feces.
2. A muscle biopsy reveals the encysted larvae. They are found near the tendon attachment.
3. A leukocytosis is present, and eosinophilia is marked.
4. The intradermal test with Trichinella-larva antigen is positive after two weeks.
5. The precipitin test is positive after four weeks.

TREMATODA

Clonorchis edemicus:

1. The opercular ova, with the shape of an electric light bulb, are found in the feces.
2. When jaundice is present, the icteric index is increased, and the direct van den Bergh test is positive.
3. Eosinophilia may be increased.

Fasciolopsis buski:

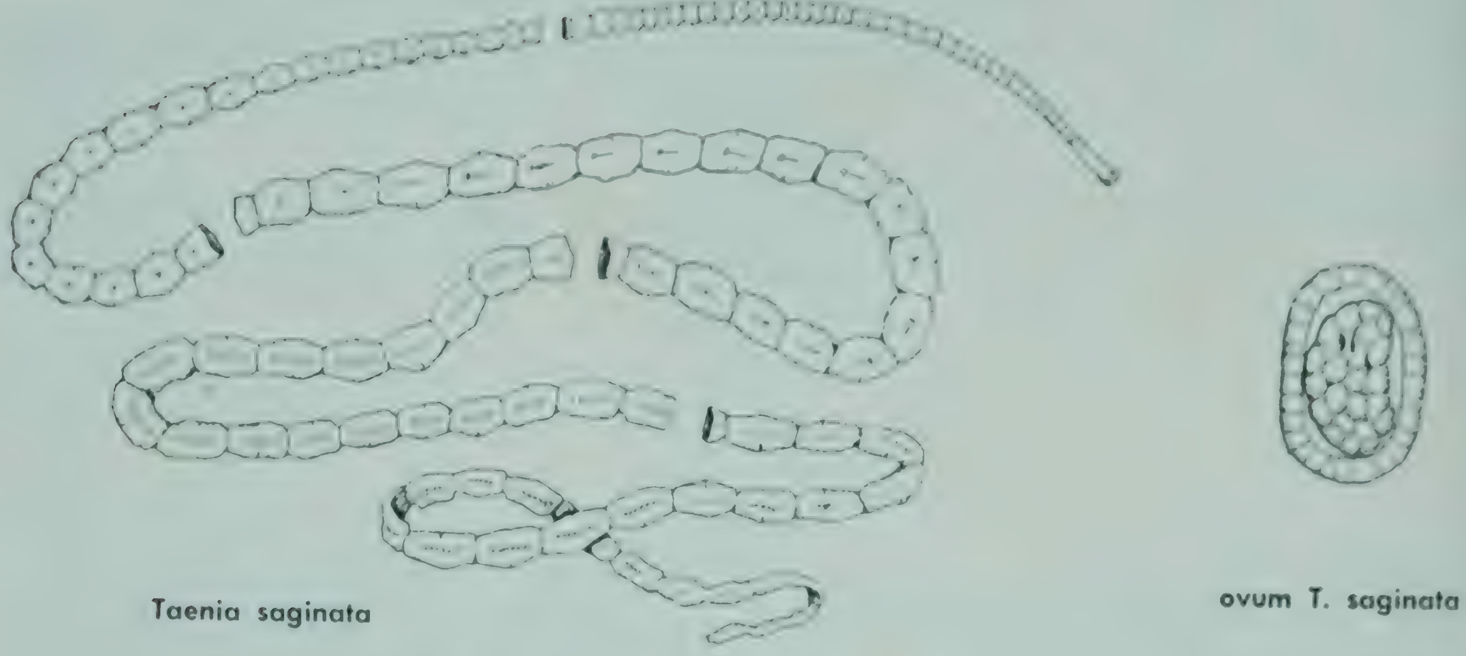
1. The brown, opercular, oval eggs or the adult flukes are found in the feces.
2. A leukocytosis is noted.
3. There is an absolute eosinophilia.

Fasciola hepatica:

1. This is rarely a parasite in man.
2. The diagnosis is made by finding the ova in the feces. They simulate the ova of Fasciolopsis buski.
3. Eosinophilia is present.
4. Jaundice is confirmed by an increased icteric index and direct van den Bergh test.
5. There may be a positive precipitin test.

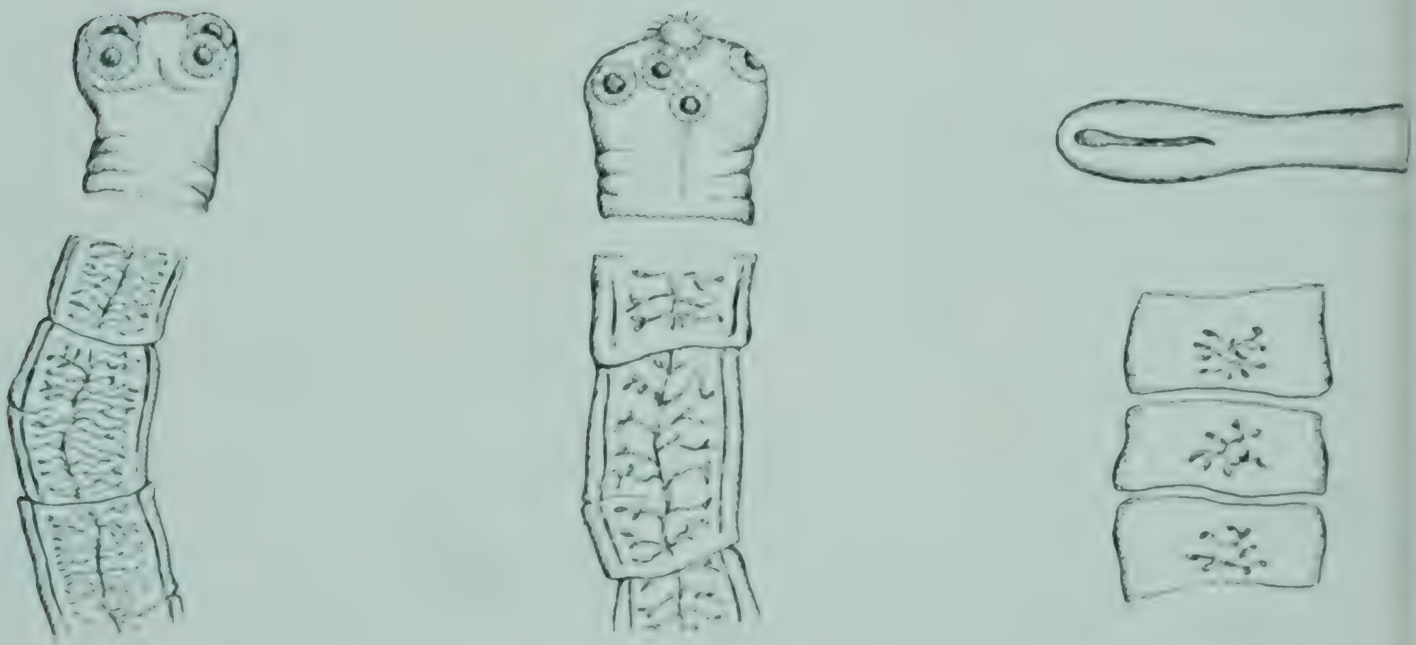
Schistosoma mansoni:

1. The oval ova with a lateral spine are found in the feces.
2. There is an eosinophilia.
3. The leukocytes are decreased.
4. There is a moderate secondary anemia.



Taenia saginata

ovum T. saginata

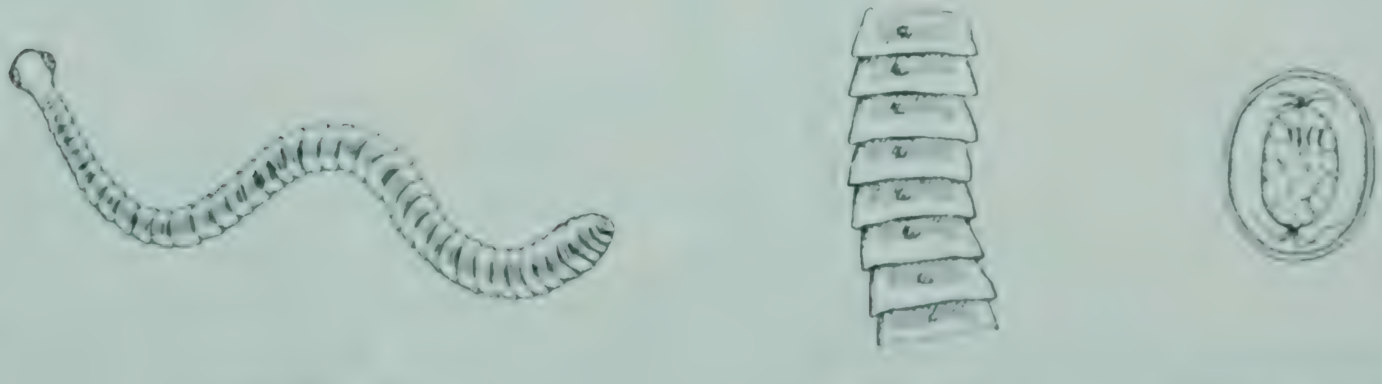


T. saginata

T. solium

Diphyllobothrium latum

Details of Heads and Uteri



Hymenolepis nana

segments

ovum H. nana

Fig. 6: Cestoda.

	5. The precipitin and complement-fixation tests may be positive.
Schistosoma japonicum:	1. Oval ova with a minute lateral spine are found in the feces. 2. Eosinophilia and anemia are observed. 3. The complement-fixation test may be positive.

CESTODA

Taenia saginata:	1. The diagnosis is made by finding the ova or segments in the feces. The segment, or proglottis, contains eighteen to thirty uterine branches, which differentiate it from Taenia solium. 2. Eosinophilia is frequently observed in massive infestations. 3. Leukocytosis is present early and is followed by a leukopenia.
Taenia solium:	1. The ova or segments are found in the feces. The mature segment has a demonstrable uterus with eight or nine branches, which distinguishes it from Taenia saginata. The ova cannot be differentiated from those of Taenia saginata. 2. Secondary anemia and eosinophilia may occur but are not constant.
Diphyllobothrium latum:	1. Examination of the feces reveals the brownish, oval, opercular ova. 2. A few cases develop a severe macrocytic anemia, which simulates pernicious anemia. 3. Eosinophilia sometimes occurs.
Hymenolepis nana:	1. Examination of the feces reveals the characteristic ova. Proglottides are sometimes found. 2. There may be a moderate eosinophilia.
Hymenolepis diminuta:	1. The diagnosis is made by finding the characteristic ova with or without proglottides. 2. Eosinophilia may occur.
Dipylidium caninum:	1. Examination of feces or smear from anus reveals the ova. Proglottides are sometimes found in the feces.

PANCREAS

Physiology:

The pancreas functions in two ways: (1) by producing an internal secretion (insulin), and (2) by producing external secretions (enzymes). Insulin is produced by the islands of Langerhans; and after being absorbed into the blood, it plays an important role in glucose metabolism. The enzymes formed by the pancreas pass into the duodenum through the pancreatic duct. The enzymes are as follows: amylase, lipase, trypsinogen, rennin, and traces of erepsin and maltase. Amylase aids in the digestion of starch. Lipase breaks fat into fatty acid and glycerol, and its activity is enhanced greatly by the presence of bile salts. Trypsinogen is converted into trypsin by enterokinase, and trypsin and erepsin from the succus entericus complete the digestion of proteins.

Diabetes Mellitus:

This disease is discussed under Endocrine Glands in Chapter 25 (p. 491).

Acute Pancreatitis:

Laboratory Examinations:

1. Serum amylase and lipase are markedly increased. The serum amylase may reach 1000 or more, expressed as the number of milligrams of glucose formed from starch by 100 cc. of serum.
2. A leukocytosis occurs.
3. Symptomatic steatorrhea develops with an increase in neutral fat in the feces. This is owing to a lack of lipase in the intestine.
4. If the function of the islands of Langerhans is interfered with, a decrease in insulin results and a subsequent hyperglycemia.
5. Blood calcium is decreased in acute hemorrhagic pancreatitis. Escaping lipase splits tissue fat into fatty acids and glycerol, and there is a great demand for calcium to unite with the fatty acids to form soap.

Carcinoma:

Laboratory Examinations:

1. Serum amylase and lipase may be increased, but not as uniformly as in pancreatitis.

2. Pratt[10] was able to study the function of the pancreas, before and after the use of secretin, in five adults with proved cancer of the pancreas. This was done by examining the duodenal contents after giving secretin. Without the use of this agent, no evidence of pancreatic insufficiency would have been observed in four of the five cases. According to Pratt, it seems proper to assume the presence of organic disease of the pancreas if low enzme values are found in the duodenal contents after the injection of secretin.
3. Jaundice may be associated with cancer of the pancreas because of obstruction of the common bile duct by the tumor. With this, the icteric index is increased; the direct van den Bergh test is positive; cholesterol and cholesterol esters are increased; the stools are acholic; and the urine contains bile but no urobilinogen.

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CHAPTER 23

Liver and Biliary Tract

The function of the liver and important liver-function tests are described in Chapter 12 (p. 226), and the abnormal findings in the bile are discussed in Chapter 5 (p. 168). The numerous functions of the liver make it impossible to employ only a single test to determine the nature of a given liver disease. Furthermore, the value of all liver-function tests is limited because of the tremendous reserve and regenerative capacity of this organ. The remaining functioning liver plus any regenerated cells must be reduced to about 20 per cent before liver-function tests will show impairment.

SELECTION OF TESTS

To evaluate hepatic disease, the clinician must understand the various functions of the liver, the particular function to be investigated, and the test applicable to this function.

Teitelbaum, Curtis and Goldhamer[1] make the following suggestions for selection of tests:

1. In patients who are jaundiced, the best tests to use for the estimation of hepatic function are the serum-protein determination (hypoalbuminemia) and the glucose-tolerance test. Invariably such patients will have had a van den Bergh test already performed.

2. In patients who are not jaundiced, it appears that the best tests to use are the bromsulfalein-dye excretion tests and the urobilinogen test. In these patients, the van den Bergh test, too, will already have been performed.

   In either situation, the other tests may well add further information regarding the degree of impairment of hepatic function.

Snell and Magath,[2] however, recommend the galactose-tolerance, cholesterol-partition, and serum-phosphatase tests in the presence of jaundice. They also feel that changes in hepatic function in the presence of jaundice can best be followed by repeated serum-bilirubin and hippuric-acid tests. In patients who are not jaundiced, these authors recommend the bromsulfalein test as the most useful.

The following tests are suggested, under the conditions as listed, in the investigation of liver disease. It is recognized that obstructive and parenchymatous jaundice cannot always be separated, since both types are present after either type has existed for several weeks. This summary is based upon reports by Osgood[3] and Giansiracusa and Althausen.[4]

LABORATORY PROCEDURES IN PATIENTS WITH JAUNDICE

A. General
   1. Icterus index or quantitative van den Bergh test (these tests determine the level of bilirubin in the serum)
   2. Qualitative van den Bergh (this test determines whether or not the bilirubin has passed through the liver)
   3. Quantitative urinary urobilinogen (increase in urobilinogen indicates that the hepatic cells are not transforming urobilinogen, formed in the intestine, into bilirubin)
   4. Urine bilirubin (the presence of bilirubin in the urine in a jaundiced person indicates an excessive amount of bilirubin in the serum, usually of the posthepatic type)
   5. Fecal urobilinogen (the absence of fecal urobilinogen indicates that no bilirubin is reaching the intestine)
   6. Alkaline phosphatase (in biliary obstruction, there is an increase in blood alkaline phosphatase, but it is not always reliable)

B. To rule out hematogenous jaundice
   1. Complete blood studies, including a reticulocyte count and fragility test (the reticulocyte count is increased, and the fragility of erythrocytes is increased in hemolytic jaundice)

C. To differentiate obstructive from parenchymatous jaundice
  1. Gastrointestinal series (this test is helpful first in suggesting a pancreatic carcinoma, second by indicating the probability of hepatic cirrhosis when esophageal varices are found, and third by calling attention to the possibility of metastatic involvement of the extrahepatic or intrahepatic biliary ducts when a carcinoma of the stomach or intestines has been found)
  2. Total protein with albumin-globulin ratio (a serum albumin of 3.5 Gm. per 100 cc. or less occurred with equal frequency in patients with obstructive and parenchymatous jaundice; however, 75 per cent of patients, in the report by Giansiracusa and Althausen,[4] with low serum albumin and high serum globlin had parenchymatous jaundice)
  3. Intravenous galactose test (according to Giansiracusa and Althausen,[4] the intravenous-galactose test[5] is helpful in differentiating the two types of jaundice; in 82 per cent of 33 cases of obstructive jaundice of less than six months duration, the blood showed less than 20 mg. of galactose per 100 cc., while 81 per cent of 26 cases of parenchymatous jaundice had more than 20 mg.)
  4. Prothrombin response to vitamin K (obstructive jaundice lowers the prothrombin level by interfering with absorption of vitamin K; with vitamin-K deficiency there is hypothrombinemia, which is corrected quickly by the parenteral administration of vitamin K [menadione]; parenchymatous jaundice produces hypoprothrombinemia by interfering with the ability of the liver to form prothrombin; consequently, in the latter, the administration of vitamin K either does not raise the prothrombin level at all or does so slowly)

LABORATORY PROCEDURES IN NONJAUNDICED PATIENTS

A. For diagnosis
  1. Bromsulfalein test (failure of the liver to eliminate bromsulfalein from the blood indicates poor hepatic function; there may be as high as 80 per cent retention in the blood; this test also shows increased retention of the dye in the blood in parenchymatous jaundice and late obstructive jaundice)
  2. Quantitative urinary urobilinogen (see A, 3, under Laboratory Procedures in Patients with Jaundice, p. 433)
  3. Thymol-flocculation and turbidity tests (this is a test for abnormal lipoglobulin; it is increased in parenchymatous disease, such as infectious hepatitis)

4. Cephalin-flocculation test (this shows the failure of protection of normal gamma globulin by an adequate amount of normal albumin; the test is positive in parenchymatous disease, as are the thymol tests)

B. Evaluation of the severity of the disease
   1. Hippuric-acid test after excluding renal impairment (when sodium benzoate is ingested, the liver produces hippuric acid, which may be measured in the urine; in four hours after ingestion of 6 Gm. of sodium benzoate, 4.2 to 5.0 Gm. of hippuric acid is excreted in the urine; in severe hepatitis, the excretion of hippuric acid is very low)
   2. Fasting hypoglycemia (the fasting blood sugar is decreased in severe liver damage, such as complete fatty degeneration or acute yellow atrophy; this is owing to the inability of the liver to store glycogen)
   3. Tyrosinuria (tyrosine and leucine crystals are found in the urine in severe liver damage; this is owing to the inability of the liver to deaminize amino acids)

C. Evaluation of the course of the disease (the following tests approach normal as the patient improves)
   1. Intravenous galactose test (see C, 3, under Laboratory Procedures in Patients with Jaundice, p. 434)
   2. Thymol-flocculation and turbidity tests (See A, 3, under Laboratory Procedures in Nonjaundiced Patients, p. 434)
   3. Cephalin-flocculation test (see A, 4, under Laboratory Procedures in Nonjaundiced Patients, p. 435)

DISEASES OF THE LIVER

Infectious Hepatitis:

Infectious hepatitis is also known as "catarrhal jaundice." Homologous-serum jaundice is possibly identical with infectious hepatitis. Infectious hepatitis is apparently caused by a filtrable virus, which has been passed from person to person[6] but has not been transmitted to animals. Hartfall[7] observed four clinical forms of the disease: (1) gastrointestinal type (53 per cent) with insidious onset, anorexia, nausea, and vomiting; (2) febrile type (30 per cent), with an abrupt febrile onset; (3) ambulatory icteric type (10 per cent), in which there were no notable general symptoms, the jaundice being discovered by another person; and (4) hepatitis without icterus (7 per cent).

Laboratory Examinations: Laboratory examinations vary somewhat with the stage of the disease. Capps and associates[8] recommend doing a small number of tests, which are frequently repeated and whose significance is well understood, rather than to use a large number of confusing procedures.

PREICTERIC PHASE:

1. Urine bilirubin is usually positive one to six days before the onset of clinical icterus.
2. The urinary urobilinogen is usually increased.
3. Thymol-flocculation and turbidity tests, and cephalin-flocculation test are positive.
4. There is a leukopenia with an increase in number of lymphocytes. Atypical lymphocytes are present.

ICTERIC PHASE:

1. The icterus index is increased and direct van den Bergh is usually positive.
2. Thymol-flocculation and turbidity tests, and cephalin-flocculation test are positive.
3. Quantitative urinary urobilinogen is increased and bile is present in the urine.
4. The bromsulfalein test is usually not helpful in this stage.

NONICTERIC TYPE:

1. Examine urine for bilirubin.
2. Perform thymol-flocculation and turbidity tests, and/or cephalin-flocculation test. These tests are positive in the active and chronic types of the disease. They become negative when the patient recovers.
3. Urinary urobilinogen is increased.
4. Albumin-globulin ratio usually shows a high serum globulin and a low serum albumin in the presence of a low total protein.
5. Determination of alkaline phosphatase and cholesterol esters may or may not be helpful.

Portal Cirrhosis:

This is the most common form of liver cirrhosis. Icterus is noted in 30 to 65 per cent of the patients and may be due either to destruction of parenchyma or compression of biliary canaliculi by fibrous tissue.

Laboratory Examinations:

1. There is characteristically an increase in urinary urobilinogen with a daily output of up to 150 mg. In cases of deep jaundice, however, with intrahepatic obstruction, little bile reaches the intestine and little urobilinogen is formed; therefore, there is a reduction in urobilinogen in both urine and feces.
2. The serum globulin is increased and serum albumin is decreased.[9]
3. The cephalin-flocculation test is usually positive.
4. Bromsulfalein is frequently retained in the presence of jaundice, so that the test has diagnostic value for cirrhosis in the absence of jaundice.
5. Peritoneoscopy and liver biopsy are important aids in establishing a diagnosis.
6. The blood may show a macrocytic anemia or, after repeated hematemesis, a hypochromic anemia.
7. The demonstration of esophageal varices by x-ray examination is helpful in establishing a diagnosis.

Miscellaneous Intraphepatic Diseases:

To avoid repetition, the following conditions are grouped together:

Necrosis from poisons
Fatty degeneration
Acute yellow atrophy
Biliary cirrhosis
Chronic passive congestion from congestive heart failure
Carcinoma of the liver

Laboratory Examinations: Laboratory examinations are not specific for the various conditions, but they do help in evaluating the damage to the liver:

1. Icterus index is increased with jaundice.
2. The direct van den Bergh test is usually positive.
3. Plasma globulin is increased and albumin is decreased.
4. The intravenous galactose-tolerance test shows more than 20 mg. of galactose per 100 cc.
5. The hippuric-acid test shows impaired liver function.
6. Urinary urobilinogen is increased.
7. The alkaline phosphatase may be increased.
8. Tyrosine and leucine are found in the urine, particularly in acute yellow atrophy.
9. There is usually a leukopenia with a lymphocytosis.

Pyogenic Abscess:

Laboratory Examinations:

1. There is usually a leukocytosis of 20,000 to 30,000 per cubic millimeter.
2. There may be a slight increase in the icteric index.

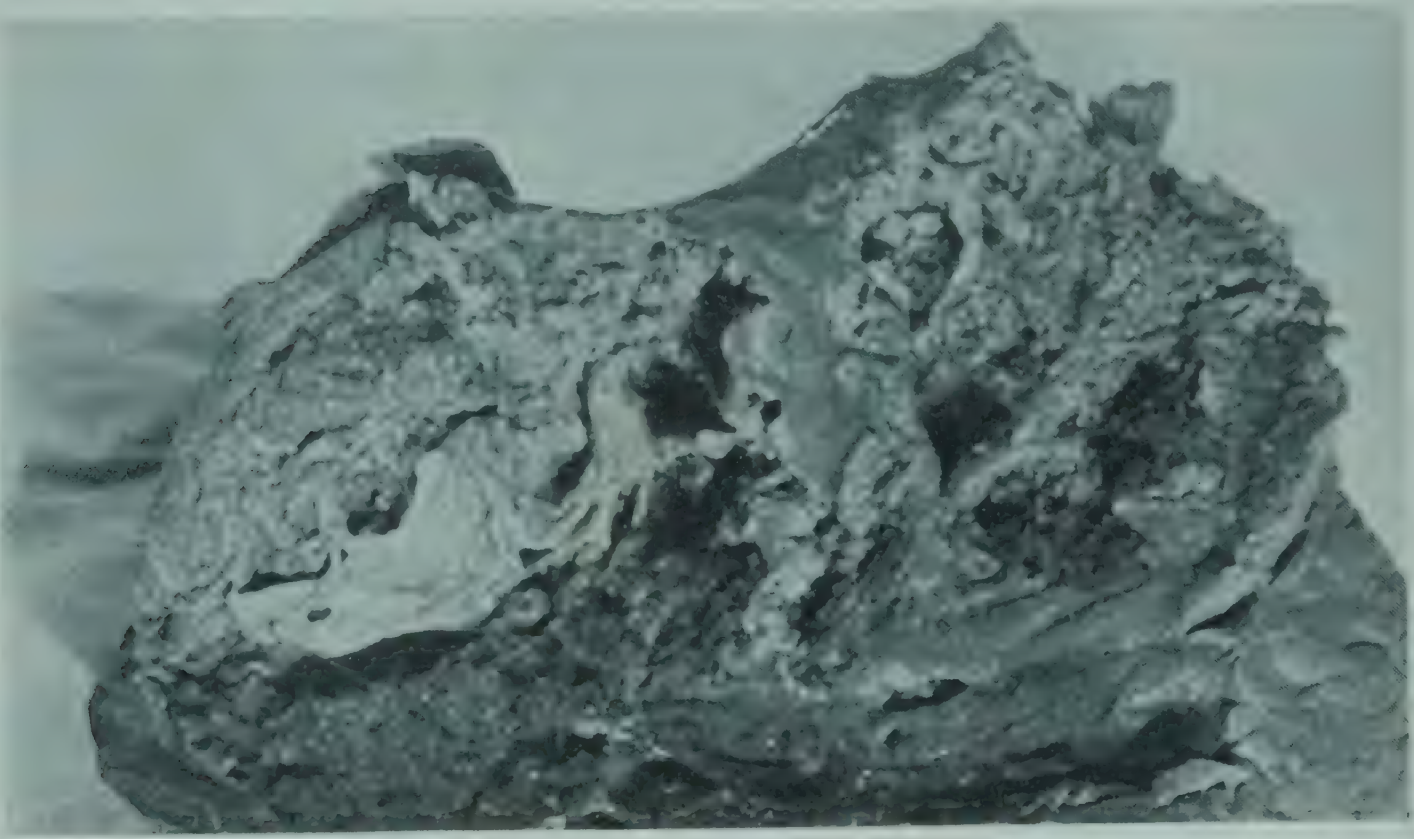

Fig. 1: Pyogenic abscess of liver.

Hemochromatosis:

This is a rare disorder of iron metabolism characterized by retention of iron. Hemosiderin and hemofuscin are deposited particularly in the skin, liver, pancreas, and other organs with the exception of the central nervous system, peripheral nerves, and parathyroid glands. Cirrhosis of the liver and pancreas is probably secondary to long-continued deposition of iron.

Laboratory Examinations:

1. Biopsy of the skin stained with potassium ferrocyanide usually reveals large amounts of hemosiderin. Similar examination of a liver biopsy also reveals hemosiderin.
2. Glucose is present in the urine, and the blood sugar is increased.
3. The icterus index is usually increased, and the direct van den Bergh is positive.
4. The total protein may be decreased, and the albumin-globulin ratio is usually reversed.

5. Blood cholesterol is frequently increased. The ratio of the cholesterol ester to total cholesterol may be reduced.
6. Thymol-flocculation and turbidity tests, and the cephalin-flocculation test are positive when liver damage is present.
7. There is usually a moderate hypochromic anemia.
8. The basal metabolic rate may be increased.
9. Some cases show levels of organic iron of the whole blood above the normal range of 50 to 52 mg. per 100 cc., according to Sacks and co-workers.[10]

Amyloidosis:

The liver, spleen, and kidneys are the usual organs where amyloid infiltration is seen. Amyloidosis occurs in chronic suppuration, such as osteomyelitis, and in tuberculosis and syphilis. The chemical nature of amyloid is not known except that it appears to be a protein.

Laboratory Examinations:

1. The Congo-red test shows a disappearance of 40 to 60 per cent or more of the dye from the bloodstream in one hour. The dye is absorbed by the amyloid.
2. In amyloidosis, Selikoff and Robitzek[11] find that biopsy of the gingiva reveals amyloid deposit in microscopic sections stained with methyl violet. It is frequently positive in those with moderate amyloidosis, including those who have negative Congo-red tests. It is useful, therefore, as a supplement to the dye test.

PARASITES

Amebic Abscess:

Laboratory Examinations:

1. The Craig complement test is positive.
2. Puncture of the liver may yield material that contains the parasites.
3. Examination of the stool may show amebas.
4. The icterus index may or may not be increased.

Hydatid Cyst:

Laboratory Examinations:

1. The intradermal skin test is positive with echinococcus antigen (cyst fluid).
2. The complement-fixation test is positive with echinococcus antigen.

Leishmaniasis:

Laboratory Examinations:

1. Examination of liver biopsy shows Leishmania donovani.
2. Examination of blood smears shows Leishmania donovani in polymorphonuclear neutrophils and in monocytes.

Flukes:

Laboratory Examinations:

Examination of bile from the duodenum may reveal ova of Fasciola hepatica, Clonorchis endemicus, or Opisthorcis filineus. They may also be found in the feces.

Schistosomiasis:

Laboratory Examinations:

1. Examination of bile reveals ova of Schistosoma mansoni, which is most likely to produce lesions in the liver. These ova may also be found in the feces.
2. The intradermal skin test and complement-fixation reactions are positive.

EXTRAHEPATIC BILIARY DISEASES

The extrahepatic biliary lesions that present diagnostic problems are those that cause obstruction at some point along the biliary tract. Unfortunately, there are no specific laboratory tests by which the nature of the obstruction can be determined. Obstruction may be caused by calculi; cancer of the head of the pancreas, the ampulla of Vater, bile ducts, or gallbladder; adhesions; kinks of the ducts; pressure on the ducts by tumors; and congenital atresia.

Laboratory Examinations:

1. The icterus index and quantitative van den Bergh are markedly increased depending on the extent of the obstruction.
2. The direct van den Bergh test is positive.
3. Urine bilirubin is positive.
4. Fecal urobilin is absent.
5. Alkaline phosphatase is usually increased.
6. Plasma proteins are normal, and there is no reversal of the albumin-globulin ratio.

7. The intravenous galactose test shows less than 20 mg. of galactose per 100 cc. remaining in the blood.
8. Hypoprothrombinemia is corrected quickly by the parenteral administration of menadione.
9. Bromsulfalein, thymol-flocculation and turbidity, and cephalin-flocculation tests are normal until the liver is damaged.

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CHAPTER 24

The Genitourinary System

KIDNEYS

Infections of the kidneys, the nephritides, nephroses, and obstructions of the urinary tract, reflect changes in both the urine and blood chemistry, which are of diagnostic and prognostic aid to the clinician. The discussion is limited to those conditions that lend themselves to laboratory diagnosis.

INFECTIONS

Pyogenic Infections:

The majority of infected kidneys show involvement of both the parenchyma and pelvis. Such a condition is called "pyelonephritis"; and when the pelvis alone is involved, it is designated as "pyelitis."

In chronic infections, particularly with obstruction, the pelvis and calyces become dilated and filled with creamy pus. If the process continues, the kidney may be reduced to a mere bag of pus as a result of thinning of the cortex.

Infection of the kidneys may take place by ascending along the ureters, particularly as a result of obstructing lesions of the urethra and prostate. When pyelonephritis develops in the absence of low urinary obstruction, it is generally believed to be of hematogenic origin. In the presence of ureteral obstruction, the pyelonephritis is presumably of hematogenic origin. The periureteral lymphatics are believed by some (Eisendrath and Kahn,[1] and Sweet and Stewart[2]) to be the route of spread of infection from the bladder to the kidneys.

Most infections of the kidneys are caused by the Escherichia coli. The remaining infections are accounted for by staphylococci, Proteus vulgaris, Pseudomonas aeruginosa, Hemophilus influenzae, and Streptococcus faecalis. Pneumococci, gonococci and Actinomyces bovis are rare offenders.

Laboratory Examinations:

1. Bacteriological examination of the urine obtained by ureteral catheterization is of prime importance. This procedure indicates the nature of the infection; after treatment, a negative culture indicates recovery.
2. Examination of the urine reveals albumin, many pus cells, a few erythrocytes, epithelial cells, bacteria, and increased specific gravity. The volume is reduced in the twenty-four hour excretion.
3. The renal function of each kidney should be determined to find out the extent of involvement. This may be done by the phenolsulfonphthalein test.
4. There is usually nitrogen retention as evidenced by increased nonprotein nitrogen, urea nitrogen, and uric acid. Persistence of a high nonprotein nitrogen is of serious prognostic import.
5. The blood inorganic phosphate may be increased.
6. The alkali reserve is reduced in the presence of acidosis, which occurs particularly in children.

Tuberculosis:

Renal tuberculosis is the result of hematogenic spread from a primary active focus, such as the lungs. Sometimes, however, a primary focus cannot be demonstrated.

Laboratory Examinations:

1. Bacteriological examination of the urine obtained by ureteral catheterization is necessary in order to find out whether one or both kidneys are involved. An acid-fast stain of the sediment occasionally reveals Mycobacteria tuberculosis. Inoculation of the sediment from each kidney into a guinea pig establishes the presence of Mycobacteria tuberculosis more frequently than the direct smear.

2. Examination of the urine reveals albumin, sometimes many erythrocytes, and leukocytes. The presence of many leukocytes without bacteria is suggestive of tuberculosis.
3. The development of nitrogen retention and impairment of kidney function (phenolsulfonphthalein, kidney-function test) depend upon the extent of the disease.

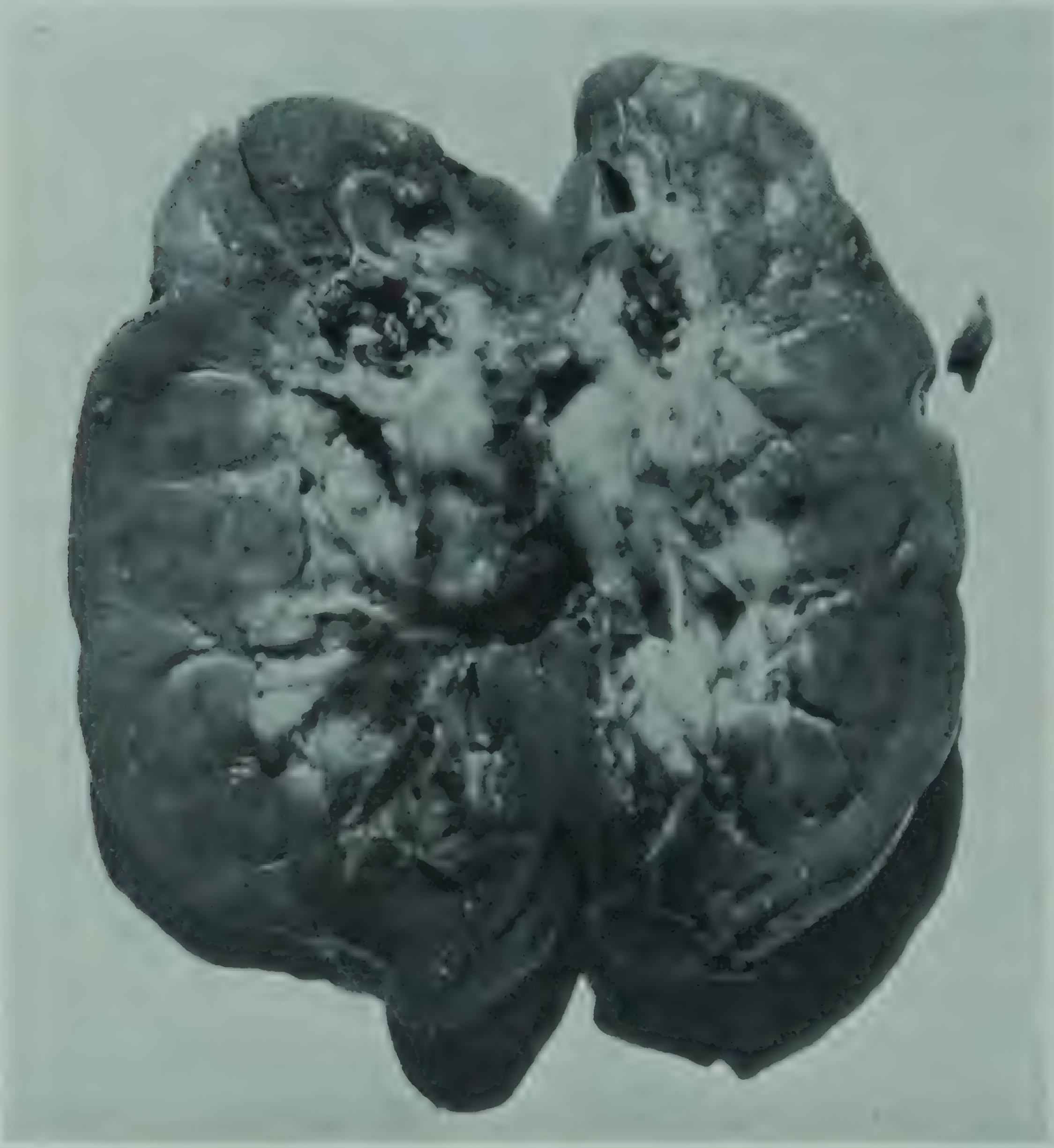

Fig. 1: Tuberculosis of kidney. Urine of this patient contained Mycobacteria tuberculosis.

NEPHRITIS

The morphological classification, devised by Volhard and Fahr,[3] of nephrosis, nephritis, and nephosclerosis is widely used because the lesions can be closely correlated with the clinical features of the disease. The following classification is based upon this concept:

1. Nephrosis
   a. Acute nephrosis

- *b*. Chronic nephrosis
    - 1. Amyloid
    - 2. Lipoid
- 2. Glomerulonephritis
    - *a*. Acute glomerulonephritis
        - 1. Diffuse
        - 2. Focal

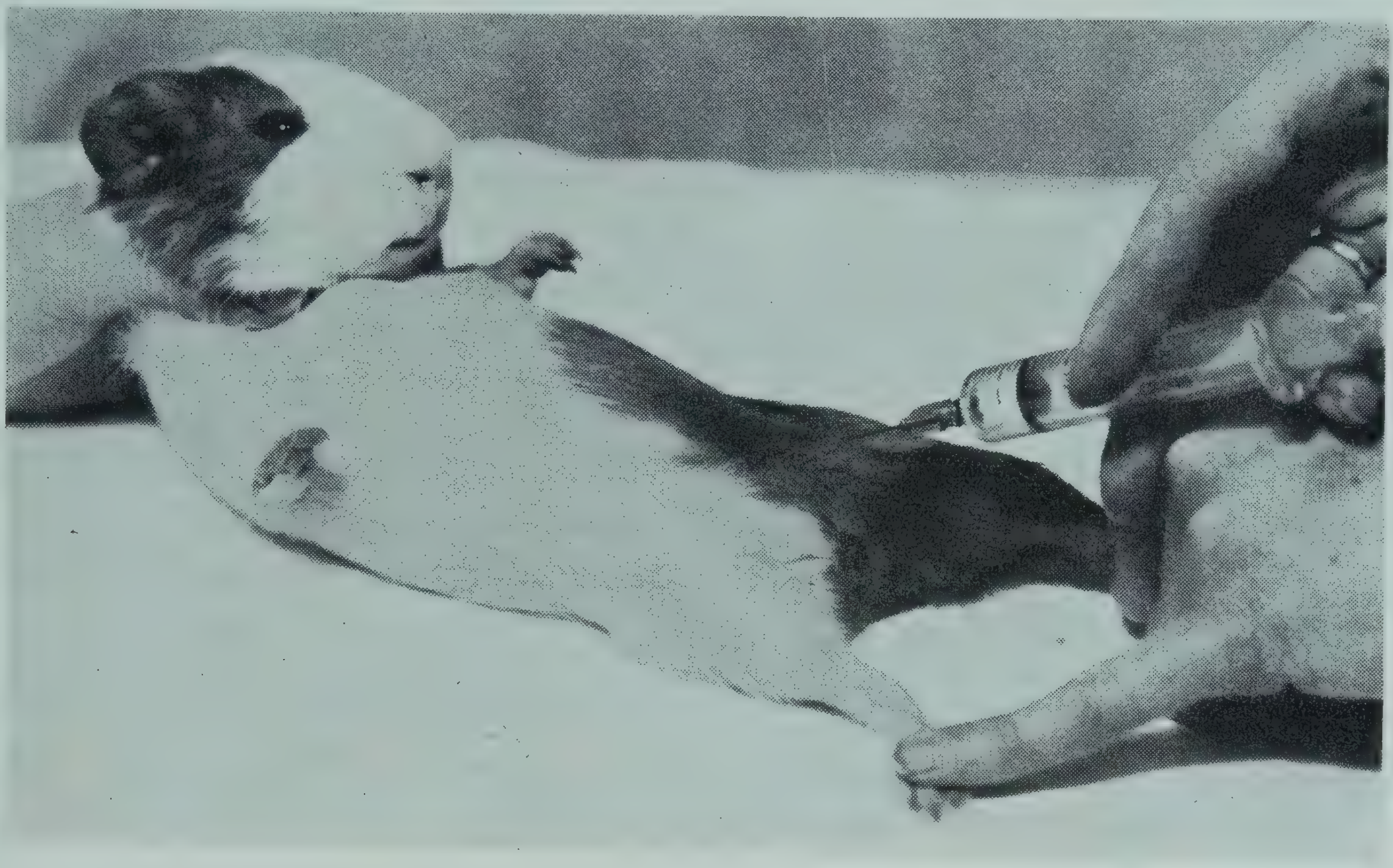

Fig. 2: Inoculation of guinea pig with urine sediment.

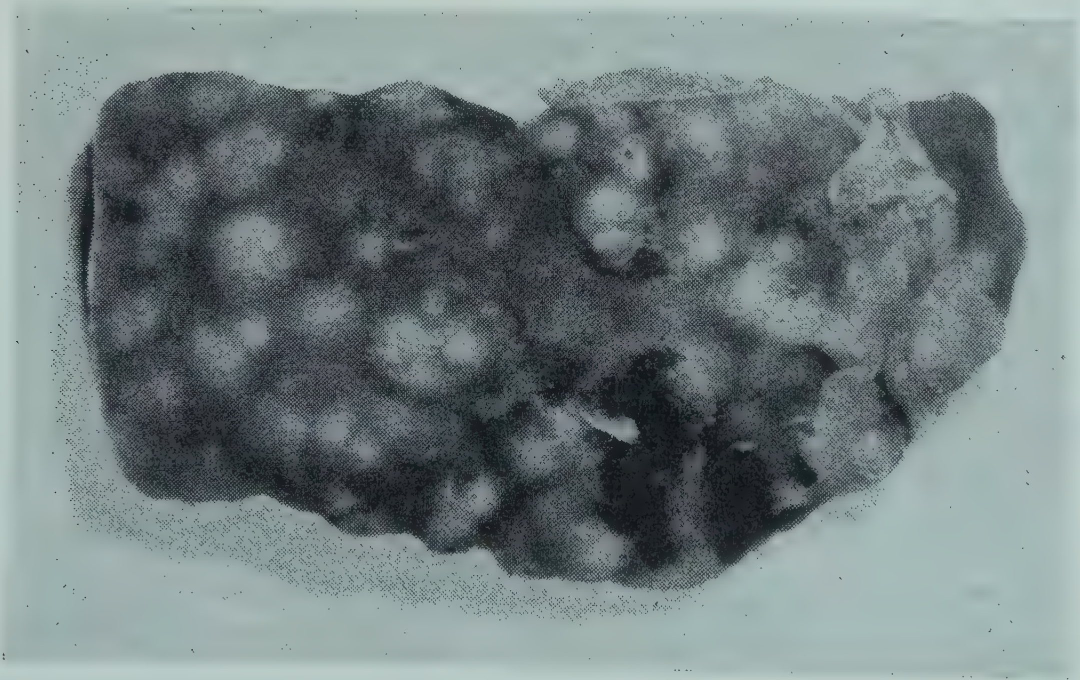

Fig. 3: Guinea-pig spleen showing tuberculosis after inoculation with urine sediment.

   - *b.* Subacute glomerulonephritis
   - *c.* Chronic glomerulonephritis
3. Nephrosclerosis
   - *a.* Arterial
   - *b.* Arteriolar

Nephrosis:

Nephrosis is a form of nephritis in which the tubules are principally involved. Although the tubules are primarily involved, glomerular changes are seen to a certain extent in all nephroses.

Acute Nephrosis: Acute nephrosis is the result of the action of toxins or injurious chemicals that are excreted by the glomeruli. These toxic agents, when in sufficient concentration in the tubules, cause cloudy swelling or hydropic degeneration of the epithelium lining the proximal convoluted tubules.

The agents that cause acute nephrosis include toxins from infectious diseases and injurious chemicals, such as bichloride of mercury, arsenic, bismuth, phenol, methyl alcohol, lead, and so on. Toxic nephrosis is also seen in such disorders as malaria, eclampsia, diabetes mellitus, obstructive jaundice, pernicious anemia, and intestinal obstruction.

LABORATORY EXAMINATIONS:

1. Urinary excretion is characterized by oliguria and, in certain cases of chemical poisoning, by anuria.
2. There is marked albuminuria.
3. Hematuria is occasionally present.
4. The total protein is reduced, with a reduction in the serum albumin.
5. Nonprotein nitrogen, urea nitrogen, and uric acid are usually increased but may be normal in the mild nephroses.
6. The blood calcium is reduced because of the loss in the urine of calcium bound to plasma proteins.
7. Blood cholesterol is usually reduced.
8. The phenolsulfonphthalein, kidney-function test may show decreased excretion.

Lower Nephron Nephrosis: Lower nephron nephrosis is the result of intravascular hemolysis from a variety of causes. These include transurethral prostatectomy, transfusion reactions, blackwater fever, crush syndrome, electrical injury to muscle, heat stroke, abortions, alkalosis from excessive vomiting, sulfonamide and quinine sensitivity, and carbon-tetrachloride poisoning.

The pathological process in the kidneys is characterized early by lipid vacuolation of the ascending limbs of Henle's loops. Hemoglobin-pigment casts are generally present in the distal convoluted and collecting tubules. Necrosis and regeneration of the epithelium of the ascending limbs of Henle's loops are noted by the fifth day. Lymphocytes are now seen between the tubules.

LABORATORY EXAMINATIONS:

1. Oliguria or anuria develops.
2. The urine is positive for albumin and hemoglobin. Red cells, white cells, and granular casts are usually present.
3. The hemochromogen level (hemoglobin, methemoglobin, hematin, and methemalbumin) of the serum is usually increased. It may range from 2 mg. to 265 mg. per 100 cc. (normal serum gives values of hemochromogen ranging from 0 to 6 mg. per 100 cc.).
4. The nonprotein nitrogen, urea, and uric acid are increased. The nonprotein nitrogen may reach 200 mg. per 100 cc. or more.
5. Acidosis develops, and the alkali reserve is reduced accordingly.
6. The white-cell count is usually increased, sometimes markedly.
7. Sometimes the patient is jaundiced, in which case the icterus index and quantitative bilirubin are increased.

Amyloid Nephrosis: Amyloidosis of the kidneys is usually part of a generalized amyloidosis in which the liver and spleen are also involved. It is associated with chronic infectious processes, particularly pulmonary and bone tuberculosis, osteomyelitis, and syphilis. The process involves first the glomeruli and later the tubules. Laboratory tests are not specific for amyloidosis of the kidneys, but they do indicate kidney damage.

LABORATORY EXAMINATIONS:

1. The Congo-red test shows rapid clearance of the dye from the blood because it is absorbed by the amyloid in the various organs. It may be absorbed by albumin excreted in the urine, which helps to clear it from the blood. This should be taken into consideration in those persons with marked albuminuria.
2. Albuminuria is variable.
3. Erythrocytes are usually absent from the urine, but hyaline, waxy, and granular casts are present in large numbers.

4. Nonprotein nitrogen, urea nitrogen, and uric acid are normal or only slightly increased.
5. Total plasma protein is frequently reduced.
6. Blood cholesterol is usually increased.

Lipoid Nephrosis: This is an exceedingly rare disease, the cause of which is obscure. However, in a certain number of cases, syphilis appears to predispose to it. It is a pure degenerative disease of the tubules, while the glomeruli, arterioles, and larger vessels are usually normal. The degenerative change consists of the deposition of an anisotropic lipoid material (cholesterol esters) in the tubular epithelium and interstitial tissue. Cloudy swelling, necrosis, or calcification of the tubular epithelium may also occur.

LABORATORY EXAMINATIONS:

1. Marked albuminuria is characteristic of the disease. The patient may lose 20 to 30 Gm. of protein per day, but this decreases with remissions.
2. Decreased excretion of urine with high specific gravity is also a constant feature during relapse.
3. Hematuria does not occur, but a few erythrocytes and leukocytes have been noted in about half of the cases. The urine sediment also contains hyaline, fatty, and granular casts, cholesterol, and doubly refractive lipoids.
4. The permeable glomeruli account for glycosuria and increased excretion of nitrogenous products.
5. Total plasma protein is decreased, and there is usually a decrease or reversal of the albumin-globulin ratio because of the loss of albumin in the urine. Plasma fibrinogen may be increased.
6. Plasma cholesterol is increased to 2.0 or more Gm. per 100 cc. Cholesterol esters appear to rise in most patients to the same extent as free cholesterol.[4]
7. According to Peters and Mann,[5] the phospholipids (lecithin, cephalin, and other phosphorus-containing lipids) are increased and parallel cholesterol.
8. The neutral fats of the blood are increased, and it has been observed that they tend to make up as much as half of the total lipids as the disease advances.
9. Serum calcium is reduced owing to the loss in the urine of the fraction bound to the plasma proteins. Since this calcium is not the diffusible type, tetany does not usually occur.
10. Renal function is normal, although later in the disease there may be a complicating glomerulonephritis, which will show evidence

of renal impairment in kidney-function tests. In this disease, as in certain other diseases, the renal-function test may show decreased excretion because of oliguria due to prerenal deviation of fluid.

11. Urea nitrogen, nonprotein nitrogen, and creatinine are usually normal. However, there may be an increase in nitrogen retention in persons with marked oliguria and edema.
12. Acidosis with decreased alkali reserve develops only after the onset of glomerulonephritis.
13. The basal metabolic rate is decreased to as much as —35.

Glomerulonephritis:

Acute Diffuse Glomerulonephritis: Although the complete etiology and pathogenesis of this type of glomerulonephritis are not clear, infectious diseases appear to play an important part in most cases. Streptococcal and pneumococcal infections are the most important underlying causes of diffuse glomerulonephritis. Damage to the glomeruli allows the escape of both protein and erythrocytes in the urine.

The results of laboratory examinations vary with the severity of the disease.

LABORATORY EXAMINATIONS:

1. Hematuria and albuminuria are characteristic findings in the urine.
2. The urine sediment, in addition to erythrocytes, contains finely granular and cellular casts, and leukocytes.
3. Oliguria with increased specific gravity of the urine is found in the acute form of the disease.
4. Nitrogen retention may or may not be present. There is, however, in most cases, an increase in the urea nitrogen and nonprotein nitrogen. Creatinine is usually normal in the acute mild form of the disease, since it is the last nitrogen compound to increase. In severe cases, however, all nonprotein-nitrogen compounds are increased, including creatinine.
5. Total plasma protein is reduced to as low as 5.5 Gm. per 100 cc. with a reduction of the albumin-globulin ratio.
6. The plasma chloride is increased because of inadequate excretion, particularly when there is decreased urinary output.
7. Kidney-function tests show evidence of renal impairment. The urea-clearance test shows a reduction before the phenolsulfonphthalein test.

8. There may be a moderate acidosis owing to inability on the part of the kidneys to excrete acid salts and to produce ammonia.
9. There may be a slight leukocytosis early in the disease.

Acute Focal Glomerulonephritis: This type of acute glomerulonephritis occurs as the embolic and toxic types. The embolic type is seen particularly in subacute bacterial endocarditis caused by Streptococcus viridans, or occasionally in other types of bacteremia or septicemia. Bacterial emboli apparently lodge in the glomerular capillaries. In the toxic type, fibrinous thrombi lodge in the capillaries, apparently owing to toxins from an infectious process outside of the kidneys.

LABORATORY EXAMINATIONS: The urine and blood findings are similar to, but usually less marked than, those of acute diffuse glomerulonephritis. However, with extensive involvement of both kidneys, the findings are similar to those of the diffuse form of the disease.

Subacute Glomerulonephritis: If acute glomerulonephritis does not clear up in six months to one year, it may be classified as subacute glomerulonephritis. This develops particularly if the primary attack was one in which there was extensive glomerular damage with marked nitrogen retention. This type blends with acute glomerulonephritis and also with the chronic form of the disease. Its separation from these other forms is somewhat arbitrary.

LABORATORY EXAMINATIONS:

1. Albuminuria persists, but it may not always be present.
2. Red cells are present in the urine sediment and should be evaluated when necessary by the Addis count. Leukocytes, and hyaline and granular casts are also found in the sediment.
3. Glucose is sometimes found in the urine.
4. Blood chloride is increased, and urinary chloride output is decreased.
5. There is usually no nitrogen retention. When it is present, the urea nitrogen and nonprotein nitrogen are only slightly increased. Creatinine is not increased.
6. Plasma protein and albumin-globulin ratio are normal.
7. Urine-concentration tests and urea clearance are usually normal. The concentration should be carried out because it is simple and also more sensitive than the urea-clearance test.
8. A mild secondary anemia may be present.

Chronic Glomerulonephritis: Chronic glomerulonephritis may be as follows: (1) a sequel of acute glomerulonephritis with or without a latent period; (2) progression from latent or subacute glomerulonephritis because of acute exacerbation, which may be the result of intercurrent infections; or (3) apparently a primary disease without a history of an acute attack, but which may be stimulated by the presence of a chronic obscure infection.

Before the uremic phase develops, clinical manifestations and laboratory findings suggest two types of chronic glomerulonephritis. One type without edema is characterized by hypertension and nitrogen retention. The second, or nephrotic, type with edema is characterized by very little nitrogen retention and slight if any hypertension. When impairment of renal function supervenes in the nephrotic type, the albuminuria diminishes. Following this, the blood proteins increase, the albumin-globulin ratio approaches normal, and lipemia diminishes. With these changes, there is decrease of edema and a change in the clinical picture from one resembling chronic nephrosis to one resembling essential hypertension. Sooner or later the nephrotic type progresses to the azotemic type without edema, and the laboratory findings change to those listed under Chronic Glomerulonephritis without Edema. These factors should be taken into consideration in connection with the laboratory examinations listed below.

LABORATORY EXAMINATIONS:

Chronic Glomerulonephritis without Edema	*Chronic Glomerulonephritis with Edema*
1. There is polyuria with low specific gravity, which becomes fixed at 1.010 to 1.012.	1. Oliguria with normal or increased specific gravity.
2. Albuminuria is slight.	2. Albuminuria is marked.
3. Red cells are found in the urine.	3. Red cells may be absent or so few as to require microscopic examination of the sediment.
4. Hyaline casts are present.	4. Hyaline and coarsely granular casts are present. Fat droplets are sometimes seen in the granular casts.

Chronic Glomerulonephritis without Edema (Continued)	*Chronic Glomerulonephritis with Edema* (Continued)
5. Urea nitrogen, nonprotein nitrogen, and uric acid are increased. Creatinine is the last to show an increase and is of serious import.	5. There is much less nitrogen retention.
6. Plasma total protein and albumin-globulin ratio are not seriously altered.	6. There is a marked hypoproteinemia with a reduction of plasma albumin to less than 2.5 Gm. per 100 cc. This loss of albumin is the cause of the edema because the osmotic pressure of the plasma proteins is so reduced that fluid flows from the capillaries into the tissues.
7. Plasma chloride is reduced because of vomiting or increased excretion in the urine.	7. Plasma chloride is increased, and chloride excretion in the urine is decreased.
8. Blood cholesterol is decreased owing to excessive excretion of cholesterol in the urine.	8. Blood cholesterol decrease occurs especially in the nephrotic type.
9. A concentration test shows fixation of specific gravity of 1.010 to 1.012. It is the first indication of renal impairment.	9. Specific gravity is normal or increased until the terminal stages when it becomes fixed.
10. Urea clearance is reduced to 50 per cent of normal or lower.	10. Urea clearance is reduced particularly in the terminal stages.
11. The phenolsulfonphthalein test shows decreased excretion, particularly in cases with high nitrogen retention.	11. The phenolsulfonphthalein test shows decreased excretion, but the decrease may be due to prerenal deviation of fluid.
12. The basal metabolic rate may be increased, particularly in the type without edema.	12. The basal metabolic rate may be increased.

Chronic Glomerulonephritis without Edema (Continued)

13. A moderate to marked secondary anemia is present. The anemia tends to parallel the nitrogen retention.

Chronic Glomerulonephritis with Edema (Continued)

13. A moderate secondary anemia is present.

Nephrosclerosis:

Arterial Nephrosclerosis: Arterial nephrosclerosis refers to sclerosis of the larger branches of the renal arteries, such as the arcuate, interlobular, or interlobar branches. Sclerosis of these vessels usually gives rise to moderate hypertension and some impairment of kidney function.

LABORATORY EXAMINATIONS:

1. The urinary excretion is characterized by polyuria and a fixation of specific gravity at the low level of 1.010 to 1.012.
2. Albumin is present in small amounts.
3. The sediment contains hyaline casts. Erythrocytes are present if there is a concomitant cardiac decompensation.
4. Plasma total protein is within normal limits. If edema develops, it is due to cardiac decompensation.
5. Nitrogen retention may be slight as evidenced by some increase in urea nitrogen and nonprotein nitrogen. With further renal impairment, there is, of course, greater increase in nitrogen retention.
6. Urea clearance, the concentration test, and the phensolsulfonphthalein, kidney-function test reveal changes indicative of the extent of renal impairment, which is present in about half of the cases.

Arteriolar Nephrosclerosis: Sclerosis of the arterioles, especially those entering the glomeruli, is more serious than arterial nephrosclerosis. Hypertension is characteristic of this form of the disease and helps to maintain adequate filtration through the glomeruli for a time. Ultimately, however, so many glomeruli become incapable of functioning that uremia supervenes. With such changes, the laboratory findings are more marked than in arterial nephrosclerosis.

LABORATORY EXAMINATIONS:

1. At first, there is polyuria with specific gravity reduced and fixed at 1.010 to 1.012. Oliguria develops later as a result of nitrogen retention or cardiac decompensation.
2. The urine shows a greater amount of albumin than in arterial nephrosclerosis.
3. The urine sediment contains hyaline and finely granular casts. Red cells are present when renal congestion takes place as a result of cardiac decompensation. Leukocytes are consistently present.
4. Total plasma protein is variable. There may be some hypoproteinemia, which becomes more marked in the later stages.
5. Nitrogen retention is present as evidenced by increase in urea nitrogen, nonprotein nitrogen, and later creatinine. Greater nitrogen retention takes place when uremia supervenes.
6. Urea clearance, the concentration test, and the phenolsulfonphthalein, kidney-function test reveal changes indicative of the extent of renal impairment. These tests usually show a more marked deviation from normal in arteriolar nephrosclerosis than in arterial nephrosclerosis.
7. A moderate secondary anemia is characteristically found in this type of nephrosclerosis.

HYDRONEPHROSIS

Hydronephrosis is overdistention of the renal pelvis with urine as a result of functional or organic obstruction somewhere in the urinary tract. If the condition persists, the pelvis and calices dilate, and the parenchyma undergoes atrophy. When the fluid is clear, it is a "sterile hydronephrosis"; and when inflammation is present, it is "infected hydronephrosis." The presence of pus in the pelvis is designated as "pyonephrosis."

The diagnosis is made by pyelograms, but laboratory procedures are of value in determining the status of kidney functions and the presence or absence of infection.

Laboratory Examinations:

1. Urine excretion is reduced or absent in bilateral hydronephrosis.
2. The urine contains a small amount of albumin.
3. The urine sediment contains hyaline or granular casts. The presence of leukocytes and erythrocytes is variable. In infected hydronephrosis, the leukocytes are numerous.

4. Culture of the urine may show the presence of an infecting organism.
5. Nitrogen retention is increased in bilateral hydronephrosis with oliguria or anuria. In unilateral hydronephrosis, urea nitrogen, nonprotein nitrogen, and creatinine are normal unless nephritis is present.
6. Blood phosphorus is frequently reduced.
7. Blood cholesterol and cholesterol esters are usually reduced as are fatty acids and phospholipids.
8. The status of function of each kidney should be investigated, preferably by doing a phenolsulfonphthalein test and collecting the urine from each kidney separately. In this way, the function of the hydronephrotic kidney may be evaluated.

UREMIA

Uremia is the toxic condition produced by the retention of urinary waste products in the blood. It is due to suppression or deficient secretion of urine from any cause, but the actual substance that causes the condition is not known. The concurrent acidosis and hypocalcemia undoubtedly contribute to the syndrome, but do not explain the whole picture. Becker's work[6] on the products of intestinal putrefaction in renal insufficiency may be of significance in the pathogenesis of uremia. He found that uremic symptoms paralleled the amounts of these putrefactive agents (indican, phenols, cresols, aromatic oxyacids, and so on) in the blood more closely than they do the nonprotein nitrogen. Von Monakow[7] found anatomical changes in the choroid plexus in patients who died in uremic coma. He believes that the partial breakdown of the barrier between the blood and cerebrospinal fluid to retained toxic substances marks the onset of the nervous symptoms of uremia.

Laboratory Examinations:

1. Nitrogen retention is characteristic of uremia. Nonprotein nitrogen is increased to as much as 300 mg. or more with a corresponding increase in urea nitrogen.

   Creatinine is the last to show an increase; and when this is increased, the prognosis is grave.
2. Urinary excretion is characterized by oliguria or anuria. The specific gravity is low and fixed.
3. Albuminuria is present and varies in amount.

4. The sediment contains varying numbers of erythrocytes, leukocytes, and casts.
5. Blood glucose is increased, and the glucose tolerance is decreased. The reason for this is not clear.
6. Plasma chloride is usually reduced because of vomiting, deficient chloride intake, or a shift of chloride to the red cells in an acidotic condition. When dehydration is present, however, the plasma chloride may be normal or increased.
7. The alkali reserve is reduced, indicating a state of acidosis. This is the result of vomiting, diarrhea, and inability of the kidney to produce ammonia.
8. Inorganic phosphorus is increased when acidosis is present.
9. Blood calcium is decreased because of a decrease in the diffusible serum calcium.
10. Plasma cholesterol is frequently decreased, but the total fat, fatty acid, and phospholipid are increased.
11. Kidney function is impaired as evidenced by decreased excretion of phenolsulfonphthalein down to less than 10 per cent in two hours.
12. Urea clearance is markedly decreased and may be as low as 5 per cent of normal.

SYMMETRICAL CORTICAL NECROSIS

Symmetrical cortical necrosis is the result of an anemic infarct of most of the cortex of each kidney. It is associated for the most part with pregnancy, but a small number of cases are associated with a variety of other diseases. The infarct is caused by thrombosis of most of the small arteries and veins just beneath the cortex, giving the cortex a mottled yellow to red appearance.

The patient develops oliguria or anuria and dies in uremia. At present, this disease appears to be universally fatal.

Laboratory Examinations:

1. The urine decreases in amount until the patient becomes oliguric or anuric. Albumin, red cells, white cells, and hyaline or granular casts are usually present in the sediment.
2. The nonprotein nitrogen increases rapidly and varies from 85 to 400 mg. per 100 cc.
3. Urea is also markedly increased.
4. Creatinine terminally reaches as high as 26 mg. per 100 cc.
5. If the patient becomes uremic, the alkali reserve drops consistently with the amount of acidosis.

6. There is a leukocytosis, which in some instances is 40,000 per cubic millimeter.

RENAL DWARFISM

Renal dwarfism, or renal rickets, is a disease usually beginning in childhood and associated with chronic nephritis, rarefaction of the skeleton, dwarfism, low serum calcium, and sometimes calcium deposits in the soft tissues. It is believed that the hypocalcemia in this disease is due to retention of phosphate resulting from renal insufficiency. However, since kidney disease not infrequently results from hyperparathyroidism and since parathyroid hyperplasia may

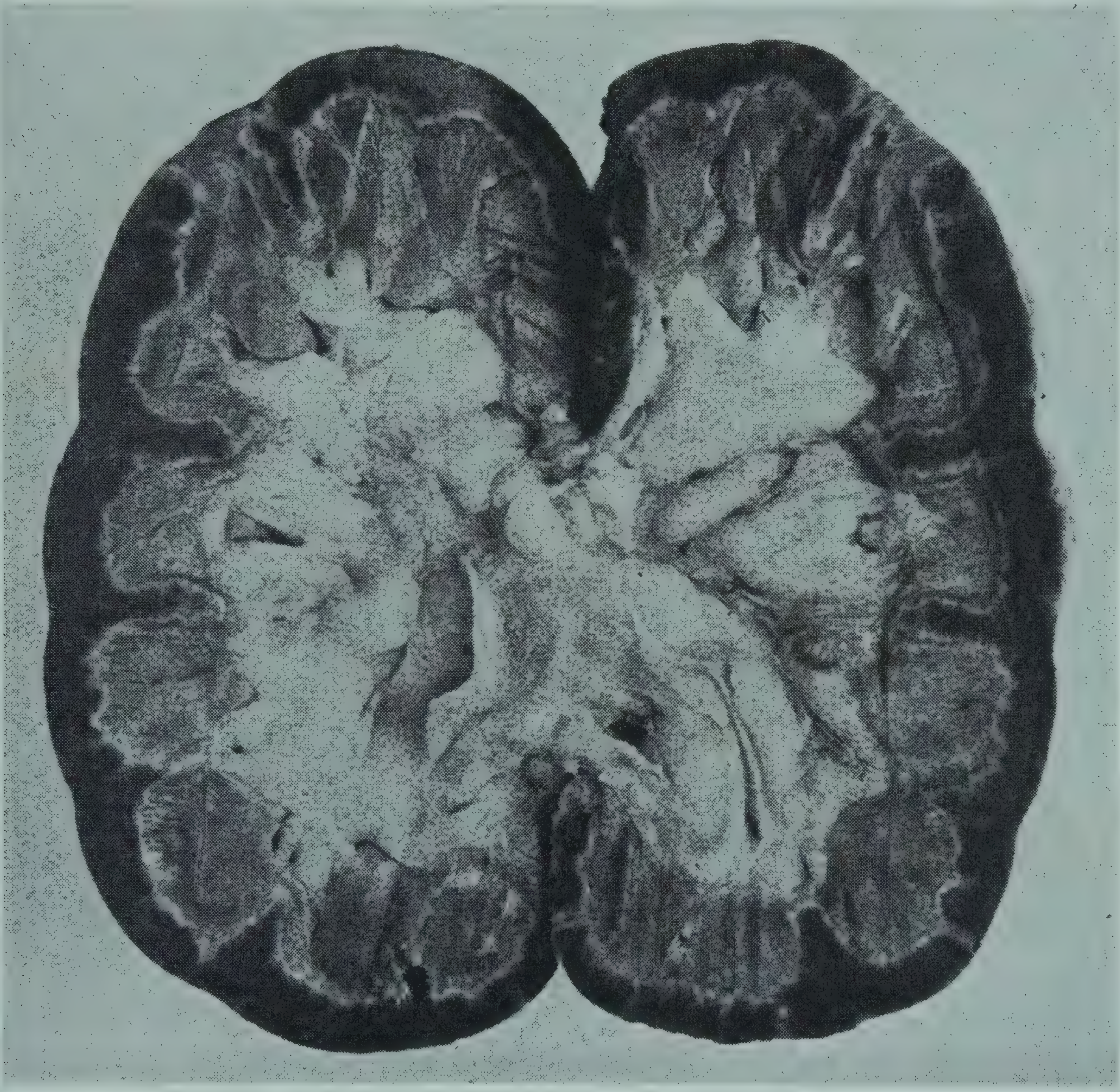

Fig. 4: Symmetrical cortical necrosis of kidney.

occur in chronic renal disease, it is very difficult in a given case to decide whether the parathyroid abnormality is a primary or secondary factor.

The prognosis is grave, and a fatal termination is the result of intercurrent infection or uremia.

Laboratory Examinations:

1. Serum calcium is markedly decreased and may reach 5 mg. per 100 cc., at which point tetany is a likely complication.
2. Inorganic phosphate is increased and may reach 15 mg. or more per 100 cc. Serum phosphatase is frequently increased.
3. Urea nitrogen and nonprotein nitrogen are increased: and in the terminal uremic phase, the values are still higher. Creatinine is also increased, particularly in the uremic stage.
4. Total protein may be reduced, particularly if there is marked albuminuria.
5. The alkali reserve is reduced to 35 volumes per cent or lower.
6. Urinary excretion is increased, and the specific gravity is reduced to 1.012 or lower.
7. Albuminuria of varying degrees is present.
8. The urine sediment contains casts and occasionally red cells.

NEOPLASMS

Neoplasms of the renal cortex include hypernephroma, papillary adenocarcinoma, malignant papillary cystadenoma, alveolar adenocarcinoma, embryonal carcinosarcoma (Wilms' tumor), fibroma, adenoma of tubules, hamartoma, hemangiona, lipoma, and myoma. Neoplasms of the renal pelvis are epidermoid carcinoma and papilloma. Tumors of the pelvis or those which impinge upon the pelvis are more likely to manifest themselves by hematuria, which is intermittent in character and variable in amount.

Laboratory Examinations:

1. Examination of the cells of the sediment from the suspected kidney by the Papanicolaou technic sometimes reveals tumor cells.
2. Hematuria is characteristic but is not pathognomonic since other conditions also produce hematuria. Red-cell casts are sometimes found.
3. Albuminuria is present with hematuria.

4. Renal function as revealed by the phenolsulfonphthalein test or urea clearance may be depressed with a tumor that involves most of the cortex. This test must be carried out separately for each kidney.
5. Nitrogen retention is variable and again depends upon the extent of the tumor.

CALCULI OF KIDNEYS AND BLADDER

Calculi of the kidneys, ureters, and bladder are important clinically because they are more common and produce symptoms. Calculi of the prostate and urethra occur but are seen much less frequently.

Etiology:

Medical knowledge of the genesis of stones is at present incomplete. There are, however, certain definite factors that undoubtedly contribute to the formation of calculi in certain persons. Deficiency of vitamin A is apparently one factor that, by causing keratinization and subsequent desquamation, forms a nucleus upon which chemicals from the urine may precipitate. Vitamin D administered in excess to rats has produced calculi made up for the most part of calcium phosphate. However, these calculi occur only when doses of Vitamin D are employed in sufficient amount to cause bone resorption and an increased concentration of calcium in the urine. Less than 1 per cent of cases of renal calculi is the result of hyperparathyroidism, but the associated abnormal blood calcium and phosphorous values make it an interesting study. Renal calculi associated with hyperparathyroidism have been studied by Albright and co-workers,[8] and by Barney and Mintz.[9]

The *p*H range of the urine appears to be a factor in the formation of calculi. Supersaturation with calcium phosphate is likely to take place in the *p*H range of 7 to 8, and this salt may precipitate out. According to Barney and Sulkowitch,[10] crystalloids, particularly calcium phosphate, may precipitate without an inflammatory process if there is prolonged urinary stasis.

The presence of infection, which allows the formation of a nidus of leukocytes, epithelial cells, fibrin, or leukocytes, is an adequate background for the formation of renal calculi when the proper chemical constituents of the urine are present in excess.

Deposits of crystals of the sulfonamides sometimes occur in the renal tubules and protrude into the pelvis. Such deposits are more likely to occur following the use of sulfathiazole and sulfapyridine.

Composition:

All calculi from the kidneys, ureters, and bladder should be analyzed, since the results may guide future therapy. Uric-acid calculi with or without sodium or ammonium urates are the most common types of stones. Since they are likely to be passed, they are not found at operation on the kidney as frequently as those composed of calcium oxalate or calcium phosphate. Renal calculi composed of cystine or xanthine are rare.

The various types of calculi are found as follows:

Kidney:	Calcium oxalate Calcium phosphate Uric acid alone or with urates Ammonium and magnesium phosphate Cystine or xanthine (rare)
Ureter:	Calcium oxalate Calcuim phosphate
Bladder:	Uric acid alone or with sodium or ammonium urate Calcium phosphate when infection is present
Urethra:	Any of above calculi

Laboratory Examinations:

The diagnosis is usually made by pyelogram or by the passage of small calculi in the urine. Laboratory examinations merely give supplementary information:

1. Urinary output is characterized by oliguria or anuria depending on the location of the calculus.
2. The irritation produced by the calculus causes hematuria of varying amounts. With it are red-cell casts, epithelial cells, and leukocytes.
3. Various types of crystals are found in the sediment. In patients with sulfonamide therapy, the characteristic crystals are sometimes found. They may or may not represent actual deposits in the renal tubules.
4. Bacteriological examination of the urine frequently reveals an infection of the kidney pelvis, bladder, or both.

5. Kidney-function tests should be carried out to determine the status of the kidneys.
6. There is usually no nitrogen retention unless there is impairment of kidney function or complete obstruction of the urinary tract by the calculus.
7. Although blood calcium, phosphorus, and alkaline phosphatase are usually normal, they should be determined to rule out other conditions, particularly hyperparathyroidism.

BLADDER

CYSTITIS

Cystitis, or inflammation of the bladder, is most commonly caused by bacteria, but it may be the result of parasitic, chemical, or thermal agents. Bacteria reach the bladder from infections of the kidneys, urethra, bloodstream, or lymphatics, or by a communication from skin, bowel, or vagina to the bladder. Conditions that predispose to cystitis are obstruction of the urethral orifice, diverticula, calculi, tumors, foreign bodies, repeated catheterizations, communication with adjacent organs, pregnancy, cystocele, and paralysis of the bladder muscle.

The organisms most commonly found in cystitis are Escherichia coli, staphylococci, streptococci, and Proteus vulgaris. Rarely, cystitis is caused by gonococci, pneumococci, or the Mycobacterium tuberculosis. Pseudomonas aeruginosa, Neisseria catarrhalis, Gaffkya tetragena, and Aerobacter areogenes may be found in combination with one of the above-named organisms.

Laboratory Examinations:

1. The urine is cloudy owing either to pus cells or bacteria, or both. Sometimes, the urine is quite clear, and pus cells are found only in the centrifuged specimen.
2. Bacteriological examination of the catheterized specimen is of prime importance in order that the sensitivity of the organism to appropriate chemotherapy may be evaluated.
3. A *p*H determination of the fresh urine is important.
4. Albumin is present, but the amount varies. It is derived mostly from the inflammatory cells.
5. Hematuria occurs in some cases.
6. In cases of schistosomiasis, the ova of Schistosoma haematobium may be found in the freshly voided urine.

NEOPLASMS

Bladder neoplasms are papillary carcinoma, transitional-cell carcinoma, epidermoid carcinoma, adenocarcinoma, colloid carcinoma, sarcoma, papilloma, fibroma, myxoma, angioma, rhabdomyoma, teratoma, and dermoid. Tumors in the bladder lend themselves to biopsy through the cystoscope.

Laboratory Examinations:

1. There should be microscopic examination of a biopsy of the tumor.
2. Hematuria is common, but it is not pathognomonic.
3. With hematuria, albumin is always found.

GENITALIA

GONORRHEA

Acute gonorrhea occurs as an urethritis in both sexes. In adult females, Bartholin's glands, Skene's glands, the vagina, the cervix, and the tubes may also be involved. In young girls and female infants, gonorrheal vaginitis is particularly contagious. These patients should, therefore, be carefully isolated.

In the male, the anterior and posterior urethra, prostate, seminal vesicles, and even the epididymis may be involved. Complications in the male include arthritis, endocarditis, periurethral abscess, myositis, tenosynovitis, bursitis, thrombophlebitis, and septicemia.

Complications in the female are less common and include arthritis, perioophoritis, periappendicitis and perirectal abscess. Septicemia and endocarditis are extremely rare.

Laboratory Examinations:

1. Bacteriological examination of the urethral discharge or other discharge is of paramount importance. If the smear is negative, a culture should be made on chocolate agar and incubated in 10 per cent carbon-dioxide tension.

   In examining smears from the male urethra, staphylococci and gram-negative diplococci other than gonococci are frequently present. In the vagina, Neisseria catarrhalis, staphylococci, streptococci, pneumococci, Corynebacterium diphtheriae, Bacillus döderleini, and other Lactobacilli constitute the normal flora. Neisseria catarrhalis and other gram-negative diplococci must be differentiated from gonococci.

2. The gonococcal, complement-fixation test is often positive in pelvic and systemic complications. However, a negative test does not rule out the disease.

SYPHILIS

Primary and sometimes secondary syphilitic lesions are found on the genitalia of both sexes. Practically all genital lesions are the result of sexual contact. Infectiousness is greatest during the primary and secondary stages when active cutaneous and genital lesions are present.

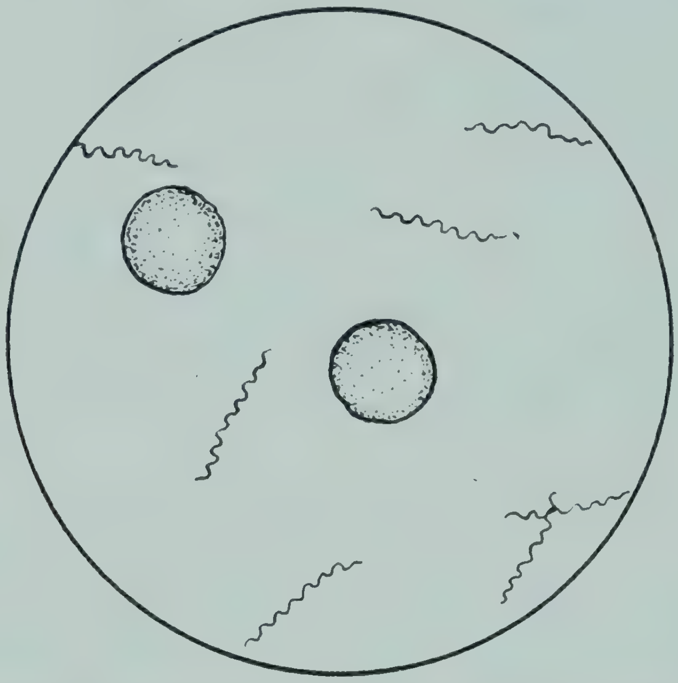

Fig. 5: Treponema pallidum, the causative agent of syphilis.

Any ulceration or abrasion of the genitalia or adjacent skin of either sex should suggest the possibility of syphilis. A darkfield examination should be done as early as possible in order that treatment may be instituted.

Laboratory Examinations:

1. Darkfield examination of serous exudate from the genital lesion should be done immediately after seeing the patient. The examination may be negative in lesions that have been treated with chemicals and in lesions that have started to heal. Aspiration of a groin lymph node may yield a positive darkfield under these conditions.
2. Flocculation and complement-fixation tests are negative for the first ten days or two weeks. After this, these tests are positive in almost 100 per cent of cases. If the darkfield is negative, it is advisable to do a serological test at that time. If the serological test is negative, it should be repeated in two or three weeks before excluding syphilis.

CHANCROID

Chancroid, or soft chancre, is a genital lesion caused by Hemophilus ducreyi. It has an incubation period of three to seven days. It may be only a local lesion, or it may involve the inguinal glands. It is frequently difficult to differentiate it from a chancre; sometimes, it may be a mixed chancroid and syphilitic lesion. In view of this, chancroids should be examined by darkfield to rule out syphilis.

Laboratory Examinations:

1. Make bacteriological examination including smear and culture of the lesion and, if necessary, the inguinal glands. The Hemophilus ducreyi may be demonstrated by these procedures.
2. A darkfield examination is important to rule out syphilis.
3. An intradermal test of a heat-killed suspension of the Hemophilus ducreyi is of value in diagnosing a chancroid. Unfortunately, the test is not positive until after the lesion has been present for one to two weeks.

LYMPHOPATHIA VENEREUM

This is a venereal disease caused by a filtrable virus. It occurs mostly among Negroes, especially in the southern part of the United States. It appears as a small lesion on the genitalia which soon heals. The first symptoms are usually swollen groin lymph nodes or buboes. The skin becomes tense and shiny over these nodes, and they ultimately break down. Stricture of the rectum is a complication in either sex. Ulcerations of the vagina and elephantiasis occur in women.

Laboratory Examinations:

1. The Frei intradermal test is of great value in establishing a diagnosis. Of course, the test will be positive if the patient has had a previous attack of the disease.
2. The complement-fixation test, using the virus, is of some help in diagnosis. It is positive in almost all active cases, but it is also positive in about 8 per cent of normal persons and in half of those with other venereal diseases. For this reason, other venereal diseases must be ruled out. However, the patient with a positive test either has the disease or has had it in the past.
3. Material from a bubo may be inoculated into the brains of mice. They develop convulsions and muscular weakness in about a

week. Examination of the brain shows perivascular lymphocytes and endothelial cells.

4. Although the virus may be cultivated, this is not a practical diagnostic procedure.

GRANULOMA INGUINALE

This disease is thought to be caused by Donovan's bodies, which have somewhat the appearance of the genus Leishmania. The early intact nodule becomes a superficial progressive ulcer. It finally involves the groins, perineum, and mesial surfaces of the thighs. Of course, other venereal diseases must be ruled out by appropriate means.

Laboratory Examinations:

1. Smears from the ulcerating buboes stained with Wright's stain will show the Donovan's bodies. The bodies occur characteristically within the large mononuclear cells, but they may be seen outside the cells. Donovan's bodies are encapsulated in chronic cases; but in acute cases, the capsules may not be present.
2. Darkfield examination, serological tests, and skin tests should be done to rule out syphilis, lymphopathia venereum, and chancroid. Granuloma inguinale may exist concurrently with any of these.

VENEREAL FUSOSPIROCHETOSIS

This is an infection of the genitalia from spirochetes and fusiform bacilli, apparently of the same type as those which cause Vincent's angina. It is apparently transmitted from the mouth to the genitalia and is associated with lack of cleanliness. It occurs mostly in male Negroes.

Laboratory Examinations:

1. Properly stained smears contain large numbers of fusiform bacilli and coarsely coiled spirochetes.
2. Of course, a darkfield examination and serological examination are advisable if the Vincent's organisms cannot be found. A serological test for syphilis should be done in any event.

TRICHOMONIASIS

One form of vaginitis is caused by Trichomonas vaginalis. It causes a profuse foamy discharge with burning and itching. There is a diffuse redness of the vagina or, in some cases, superficial ulcerations of the labia and thighs.

Laboratory Examinations:

A moist preparation from the vagina examined microscopically reveals the characteristic pear-shaped bodies with moving flagellae.

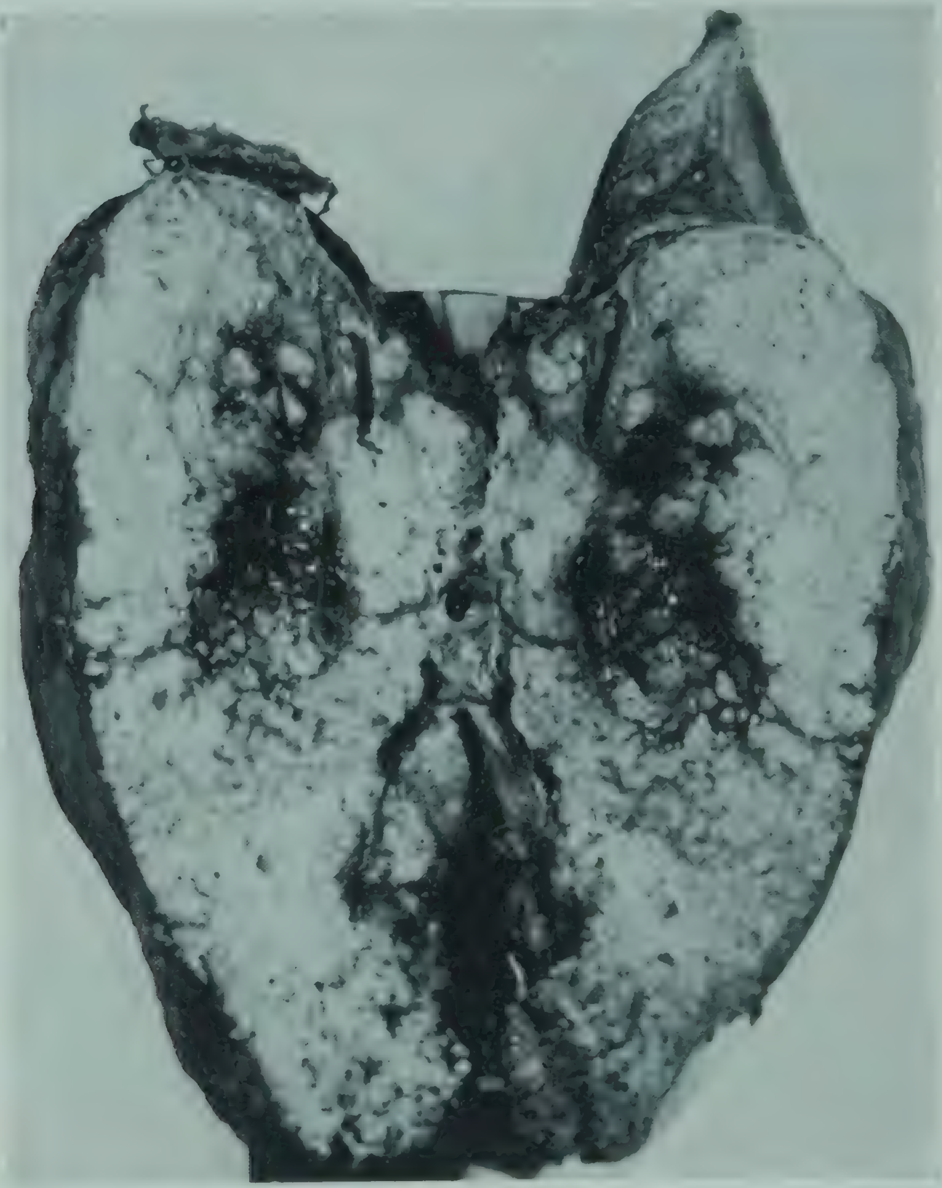

Fig. 6: Adenocarcinoma of uterus. Vaginal smear was positive for malignant tumor cells.

A convenient way of transporting material from the patient to the laboratory is in a medicine dropper. The moist preparation can then be set up and examined.

FUNGOUS VAGINITIS

Candida albicans may be found as a saprophyte in a normal vagina. It may also cause a vaginitis with a thin watery discharge and itching that is extremely disagreeable.

Laboratory Examinations:

Examination of a moist preparation mounted in potassium hydroxide will reveal the fungus. Trichomonas vaginalis should be ruled out at the same time.

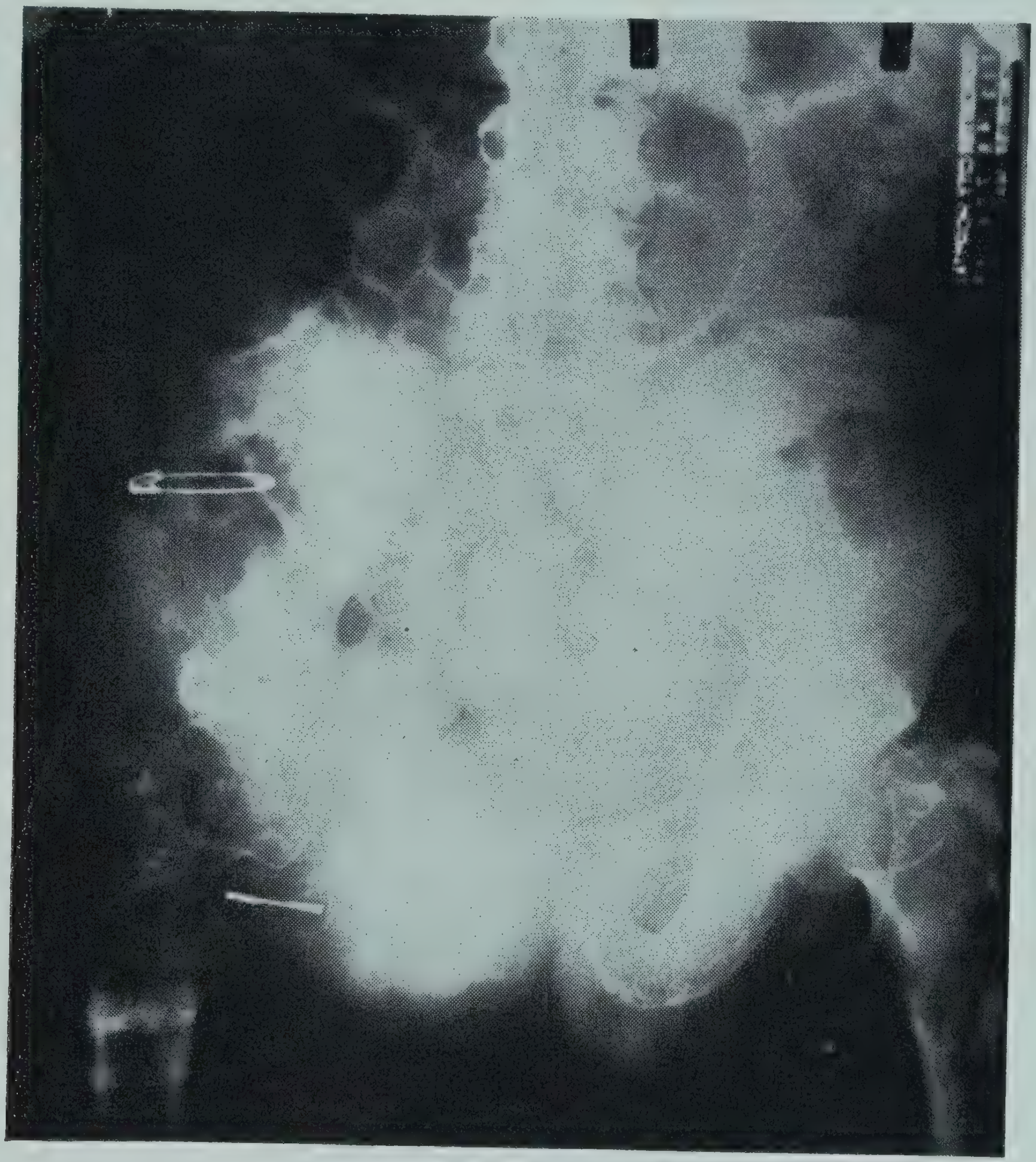

Fig. 7: Pelvis showing metastases from carcinoma of prostate.

NEOPLASMS OF THE GENITALIA

External Genitalia:

Tumors of the external male or female genitalia that are visible can be excised, or a specimen can be taken for biopsy.

Cervix and Uterus:

Likewise a specimen of the cervix for biopsy may be taken; or uterine curettage may be performed for the diagnosis of malignancy of these organs, but this requires an anesthetic and a surgeon's time.

Papanicolaou's work[11] on the cytologic diagnosis of cancer from smears taken from the posterior vaginal vault has made it much easier for the large-scale diagnosis of cancer of these organs. Material is procured with a long, slightly bent pipet, which is equipped with a bulb. It is smeared upon a slide, which is dropped into a mixture of equal parts ether and 95 per cent ethyl alcohol before it dries. After fixing for a half hour or longer, it is stained and examined for tumor cells, which may occur singly or in sheets. A knowledge of the normal cells of the vagina is, of course, necessary in order to recognize the tumor cells.

In carcinoma of either the cervix or uterus, tumor cells are shed and accumulate in the vagina. They are mixed with normal, desquamated, keratinized, epithelial cells of these organs and can be detected after proper staining.

Carcinoma of Prostate:

In addition to transurethral biopsy, certain laboratory procedures are helpful as corroborative evidence of bone-marrow metastases and of activity of the metastases.

Laboratory Examinations:

1. The blood-acid phosphatase is usually greatly increased because metastatic prostatic cancer maintains the ability to form this enzyme.
2. The alkaline phosphatase is increased when the bone lesions are osteoplastic. It is not increased when the bone lesions are osteolytic.

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CHAPTER 25

Endocrine Glands

The products of the endocrine, or ductless, glands belong to a class of physiologically active, internal secretions, or hormones. A hormone is a chemical substance that is formed by an endocrine gland, is transported by the bloodstream to another organ or tissue, and influences its activity. Except for small amounts that may be held in the endocrine glands themselves, hormones are not stored in the body. Hormones in general are stimulatory agents, but they do not stimulate the organs that secrete them, for example ovarian hormones do not stimulate the ovary.

Disorders of the endocrine gland are seen in the form of hyperfunction, hypofunction, or complete loss of function.

PITUITARY GLAND

ANTERIOR LOBE

The anterior lobe of the pituitary gland is considered to be the master gland of the body. This lobe appears to have at least eleven physiological effects.[1] Six of these extracts have been prepared as follows: (1) the growth hormone, (2) the thyrotropic hormone, (3) the adrenotropic hormone, (4) and (5) the gonadotropic hormones, and (6) the lactogenic hormone.

The remaining effects, for which separate hormones have not yet been isolated, are as follows: (7) ketogenic, (8) insulin antagonizing, (9) diabetogenic, (10) parathyrotropic, and (11) pancreatropic.

The physiology of the pituitary gland may be studied indirectly by noting the effects of its removal. The chief effects[1] of removing the pituitary gland are as follows: (1) arrested growth; (2) atrophy of the gonads and indirectly of the accessory sex organs; (3) suppression of milk secretion and involution of mammary glands; (4) atrophy of thyroid and suprarenal cortex, and probably degenerative changes in the parathyroids; (5) lowered metabolic rate; (6) hypoglycemia and increased sensitivity to insulin; (7) depression of spontaneous activity; and (8) diminished resistance to infections and shock.

HYPERFUNCTION

Hyperpituitarism is usually the result of change of function or hyperplasia in the form of adenomas. When this involves the eosinophilic cells of the anterior pituitary, acromegaly or gigantism results; and when it involves the basophilic cells, pituitary basophilism (Cushing's disease) results.

Acromegaly:

This is a form of hyperpituitarism that manifests itself by overproduction of the growth hormone by an adenoma of the eosinophilic cells during adult life. The condition is characterized by the following: (1) overgrowth of the bones of the hands, feet, and face; (2) thickening of the tissues of nose, lips, forehead, and scalp, and general overgrowth of body hair; (3) atrophy of the gonads and suppression of the sexual function, with amenorrhea in females and impotence in males; and (4) enlargement of the viscera (the tongue, lungs, thymus, heart, liver, and spleen) are enlarged; and the thyroid, parathyroids, and adrenals, which are stimulated by the pituitary hormones, may be hypertrophied. The diagnosis is usually suspected from the physical appearance, but laboratory examinations are confirmatory.

Laboratory Examinations: Examinations carried out during the active phase of the disease are as follows:

1. The basal metabolic rate is usually increased from 10 to 70 per cent.
2. Hyperglycemia and glycosuria are usually present. Sugar tolerance is decreased as in diabetes mellitus. This is probably evidence of the diabetogenic factor.
3. Iodine tolerance and blood iodine are increased, as in the excretion of iodine in the urine.
4. Blood calcium is decreased, and urinary calcium excretion is increased. According to Scriver and Bryan,[2] this is responsible for osteoporosis in certain cases.
5. Nonprotein nitrogen and uric acid are sometimes increased.
6. Blood cholesterol and lipids are usually decreased.
7. A secondary anemia of the hypochromic normocytic type sometimes develops.
8. Along with other evidences of increased function, there is increased urinary excretion.
9. Albumin and casts are frequently present.
10. Specific dynamic action of protein is not changed, according to Cushing and Fulton,[3] while Schrire[4] found it increased with increased blood and urine creatinine.
11. Serum phosphorous levels are increased before treatment, according to Hurxthal.[5] Too much emphasis should not be placed upon a single determination.

Gigantism:

This condition is also due to an adenoma of the eosinophilic cells of the anterior pituitary, but it occurs in young persons before ossification is completed. A general overgrowth of the skeleton takes place, and giants 7 or 8 feet tall result. The viscera are not enlarged, but the features simulate those seen in acromegaly. The diagnosis is usually evident from the physical appearance, and consequently laboratory tests are not usually necessary.

Laboratory Examinations: The laboratory examinations during the active stage are essentially the same as those of acromegaly. Later, the condition reverts to a hypopituitary state, and the laboratory findings are, in general reversed.

Pituitary Basophilism (Cushing's Disease):

This is thought to be caused by an adenoma of basophilic cells. The disease is characterized by the following: (1) obesity of abdomen, face, and buttocks, but not the limbs; striae due to distention are present over the lower abdomen; (2) pigmented skin and overgrowth of hair; (3) cyanosis of the face, hands, and feet; (4) loss of mineral from the bones, sometimes involving the dorsal vertebrae and causing kyphosis; (5) hypertension; (6) decreased sexual function; (7) glycosuria and hyperglycemia; and (8) atrophy of ovaries or testes.

Pituitary basophilism is sometimes modified by the presence of adrenal-cortical tumors.

Laboratory Examinations:

1. The basal metabolic rate is usually increased.
2. Prolan A and B are increased in the urine. Estrin is usually absent. However, when there is increased estrin in the female or androgen in the male, it is suggestive of an associated adenoma of the adrenal cortex.
3. Hyperglycemia and glycosuria are present and are evidence of excessive secretion of diabetogenic hormone. Glucose tolerance is decreased.
4. Both serum and urinary calcium are increased, and serum phosphorus is normal. The former is due to excessive secretion of the parathyrotropic hormone.
5. Nonprotein nitrogen may be increased.
6. Increase of blood cholesterol may be present.
7. In cases not associated with adrenal tumors, the 17-ketosteroid excretion is normal (men, 15 mg. per day; women, 10 mg. per day) or only slightly increased. When a functioning cortical tumor of the adrenal complicates the condition, the excretion is increased and ranges from 40 to 288 mg. per day.

HYPOFUNCTION

Hypofunction of the anterior pituitary may be a late manifestation of the hyperfunction diseases described above. It may also be a primary manifestation as the result of a variety of causes, such as hemorrhage, infection, congenital anomalies, syphilis, or tumors involving the anterior lobe. Hypofunction brings about clinical conditions that are quite the opposite of those caused by hyperfunction, and the laboratory findings are usually reversed.

Dwarfism:

One form of this condition results from deficiency of the growth hormone of the anterior pituitary. Persons with this form of dwarfism are usually under-developed sexually, but they do not show deformity or mental inferiority. During infancy and childhood, the ossification centers appear normal, and dentition is not delayed. Many show precocious senility with loose wrinkled skin. The diagnosis can be made on the basis of the physical examination.

Laboratory Examinations:

1. Fasting blood glucose is reduced and there is increased tolerance to glucose.
2. The basal metabolic rate is decreased.
3. The blood chloride is decreased, and excretion of chloride in the urine is increased.

Pituitary Cachexia:

This condition was first described by Simmonds[6] in 1914, and it is frequently designated as "Simmonds' disease." It is due to deficiency of the anterior lobe, which results from atrophy, fibrosis, hemorrhage, cystic degeneration, neoplasm, or thrombosis. The disease is characterized by marked emaciation and general wasting of the body. The skin becomes wrinkled and dry because of loss of fat. The hair is sparse and dry.

Deficiency of function of the anterior pituitary may occur in a milder degree than that of classical pituitary cachexia. The clinical and laboratory findings, therefore, may suggest varying degrees of severity of the disease. Anorexia nervosa is the most difficult condition to differentiate from pituitary cachexia.

Laboratory Examinations:

1. Excretion of 17-ketosteroids in the urine is reduced to from 2 to 0.5 mg. per day, depending on the severity of the disease. This reduction in 17-ketosteroids is due to the fact that pituitary insufficiency induces adrenal-cortical and testicular insufficiency.

   In anorexia nervosa, the excretion of 17-ketosteroids is usually higher than in pituitary cachexia. It ranges from 15 to 2 mg. of excretion in the urine per day.
2. The basal metabolic rate is markedly reduced.
3. There is usually hypoglycemia and increased tolerance to glucose. However, a low renal threshold may cause glycosuria.
4. There is marked sensitivity to insulin. The usual dose required to produce a reaction is 0.1 unit per kilogram of body weight. It

requires only a portion of this amount to induce a reaction in pituitary cachexia.

Fraser, Albright, and Smith[7] reported on the use of the insulin-tolerance test in panhypopituitarism and in normal persons with resulting blood-glucose levels as shown in Fig. 1.

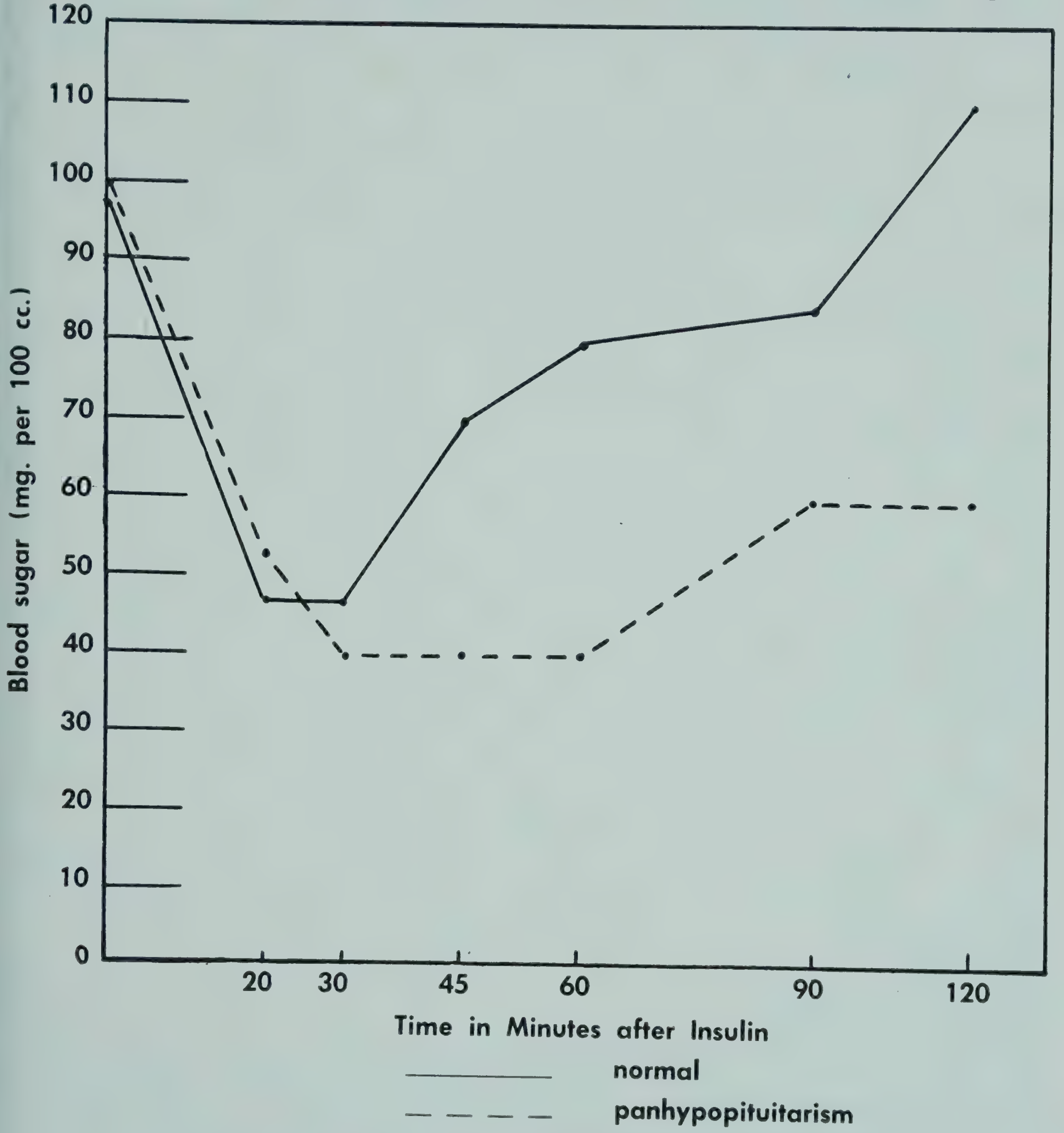

Fig. 1: Insulin-tolerance test.

The procedure consists of injecting 0.1 unit of insulin per kilogram of body weight, or better 0.1 unit of insulin per kilogram of ideal weight as determined by a height-age table. Blood samples are taken at 0, 20, 30, 45, 60, 90, and 120 minutes for the determination of glucose levels. Usually 0.01 cc. of

1:1000 epinephrine per kilogram of body weight is given after the 120 minutes. Two more blood samples are taken at 45 and 60 minutes.

It is, of course, important to watch for signs of hypoglycemia. In cases suspected of having panhypopituitarism, adrenal-cortical hypofunction (Addison's disease), or hyperinsulinism, it is advisable to give one half or one third the dose.

5. Free hydrochloric acid may be reduced or absent.
6. A severe type of hyperchromic anemia may be present.
7. The white-cell count is usually normal. The differential count frequently reveals an increase in lymphocytes or eosinophils.

POSTERIOR PITUITARY

The posterior pituitary, or neural lobe, is the source of posterior-pituitary extract, which is commonly known as "pituitrin." This material is obtained from the posterior lobes of the pituitary glands of cattle. The crude extract increases the blood pressure and respirations, exerts an antidiuretic effect, and causes contraction of smooth muscles and expansion of the melanophores.

This crude extract has been separated into two fractions: (1) *beta*-hypophamine (pitressin, or vasopressin), and (2) *alpha*-hypophamine (oxytocin). The pitressin exerts the pressor, diuretic, antidiuretic, and intestinal-stimulating effects. The oxytocin fraction is a powerful stimulant of uterine contractions. The diuretic effect of pitressin is transient, while the antidiuretic action is more prolonged. It is because of the latter action, that it is used in the treatment of diabetes insipidus. The antidiuretic action is brought about by stimulating the tubules to reabsorb water excreted by the glomeruli.

Diabetes Insipidus:

This condition is characterized by the consumption and excretion of large amounts of water. As much as 30 liters a day may be consumed in marked cases. In most cases, the administration of posterior-pituitary extract causes prompt relief of the polyuria and polydipsia. According to Biggart,[8] since a few cases are not relieved by the administration of posterior-pituitary extract, it would appear that not all cases are due to deficiency of the posterior lobe of the hypophysis. It is possible in such cases that there is a functional disturbance of the hypothalamus, but this is not yet proved. The fact that posterior-pituitary extract does not always relieve the polyuria following pituitary operations suggests that the origin may be outside the neural lobe of the hypophysis.

Since it may be confused clinically with diabetes mellitus or polycystic kidney, laboratory examinations are helpful in making a diagnosis.

Laboratory Examinations:

1. Excretion of 20 to 30 liters of urine a day is characteristic. It has a specific gravity approaching that of water, 1.005 to 1.001.
2. Injection of 10 U.S.P. units of pituitrin brings about normal concentration of urine in diabetes insipidus as evidenced by increased specific gravity. In simple restriction of fluids, the specific gravity is not appreciably increased.
3. To differentiate diabetes insipidus from hysterical polydipsia, the patient refrains from drinking water as long as possible. In diabetes insipidus, the specific gravity does not exceed 1.007, while in hysterical polydipsia it is usually above 1.012.
4. Plasma chloride is usually within normal range (570 to 620 mg. per 100 cc.), and the urinary excretion of chloride is normal whether the patient is taking posterior-pituitary extract or not.
5. The basal metabolic rate is increased, probably due to increased function of the kidneys. It returns to normal when the patient is under treatment with posterior-pituitary extract.

PLACENTA

Hormones:

The human placenta contains two groups of hormones: (1) estrone, or theelin (ketohydroxyoestrin), and estriol, or theelol (trihydroxyoestrin); and (2) anterior pituitarylike substances, or chorionic gonadotropin. The anterior pituitarylike substance has an action similar to, but not identical with, the gonadotropic substances of the anterior pituitary. The anterior pituitarylike substance of the placenta was proved to be identical with the gonadotropic principle of pregnancy urine. The Aschheim-Zondek and Friedman tests for pregnancy are based upon the presence of gonadotropic substance in the urine. See Chapter 4 (p. 148) for discussion of the pregnancy test.

OVARIES

Hyperfunction:

Hyperfunction of the ovaries is due to excessive secretion of estrin by the graafian follicles, follicular cysts, corpora lutea, or granulosa-cell tumors. The follicle-maturing hormone (Prolan A) of the anterior pituitary acts upon the ovaries. Hyperfunction of this gland can also cause increased secretion of estrin by the ovaries.

Laboratory Examinations:

1. Endometrial biopsy reveals glandular hyperplasia with or without the formation of cystic endometrium.
2. The estrogen level in the urine is increased. The normal excretion is 0.08 mg. per day at the maximum, with the minimum excretion at the time of menstruation.

Hypofunction:

Hypofunction of the ovaries may be due (1) to deficiency of the pituitary gland resulting in inability to secrete prolan A and B, (2) partial or complete replacement of functioning ovarian tissue by a cyst or nonfunctioning tumor, and (3) bilateral oophorectomy or radiation of the ovaries producing artificial menopause. The result is decreased, irregular, or absent menstruation.

Laboratory Examinations:

1. The urine shows a decreased excretion of estrin.
2. Vaginal smear shows a preponderance of leukocytes with only a few epithelial cells. With only a moderate decrease in estrin, the leukocytes and epithelial cells are more nearly equal in numbers.
3. Biopsy of the endometrium shows atrophic glands with fibrotic stroma.
4. When it is due to hypofunction of the anterior pituitary, there are decreased amounts of prolan A and B in both the blood and urine.
5. There may be an associated hypothyroidism, in which case the basal metabolic rate is reduced.

ADRENAL GLAND

The adrenal gland is composed of a central medulla, an intermediate androgenic zone, and a peripheral interrenal zone. Hyperplasia and adenomas of the interrenal zone apparently do not cause any particular syndrome. However, tumors of the androgenic zone may cause disturbance of the secondary sexual characters, which is known as the "adrenogenital syndrome." With modern knowledge, it is possible to relate these changes to the androgenic cell and to classify the syndrome into three types as given below.

Adrenogenital Syndrome:

The adrenogenital syndrome may be the result of hyperplasia, adenoma, or carcinoma of the adrenal cortex.

Juvenile Form: This disorder begins during the first two years of life. While it may occur in males, it is seen five times as frequently in females. It is characterized by masculinization, evidenced by premature muscular development, deepening of the voice, and hirsutism. Muscular development is much more evident in males. Females have enlargement of both labia majora and minora, and hypertrophy of the clitoris. In males, the penis is enlarged, but the testes are immature[9] and show no evidence of spermatogenesis.

Adult Form: This occurs in adult females and is characterized by hirsutism of male distribution, development of a bass voice, hypertrophy of the clitoris, change in body contour from female to male type, cessation of menstruation, and adiposity of the torso.

Pseudohermaphroditic Type: There is frequent critical hypertrophy in cases of pseudohermaphroditism. These persons have female reproductive organs with secondary male characteristics. These characteristics may be the result of androgenic activity of the adrenals, which may be the result of a congenital maldevelopment.

Laboratory Examinations:

1. The 17-ketosteroid excretion in the urine is increased to as much as 100 mg. per day as compared with a normal of from 7 to 10 mg. per day.
2. Pregnandiol excretion in the urine is increased from a normal of 5 to 10 mg. to as much as 30 mg. per day.
3. There is increased excretion of androgens, particularly in the pseudohermaphroditic type.

4. Blood sodium is increased, and potassium is decreased.
5. Total blood cholesterol is usually increased.

HYPOFUNCTION

The outstanding condition representing hypofunction of the adrenals is adrenal-cortical hypofunction (Addison's disease). This is caused by decreased function of the adrenal cortex because of tuberculosis, amyloidosis, syphilis, atrophy, hemorrhage, or infarction. Patients with Addison's disease have asthenia, hypotension, pigmentation of skin and mucous membranes, subnormal temperature, anorexia, nausea, vomiting, syncope, cessation of menses, amenorrhea, and atrophy of the testicles in males.

Laboratory Examinations:

1. In the male, the 17-ketosteroids excreted in the urine fall to from 1.2 to 6.4 mg. per day, which represents the output of the testicles. In females, the level falls almost to zero.
2. The test introduced by Robinson, Power, and Kepler[10] is helpful in the diagnosis of Addison's disease:

   Procedure I (based on volume of urine): On the day before the test, the patient eats three ordinary meals but omits extra salt. He is requested not to eat or drink anything after 6 P.M. Until this time, he may drink water as desired. At 10:30 P.M., he is requested to empty his bladder and discard the urine. All urine that is voided from then on, until and including 7:30 A.M., is collected. The volume of this urine is measured and saved for chemical analysis if this should be necessary later. Breakfast is omitted. The patient is asked to void again at 8:30 A.M., and immediately thereafter he is given 20 cc. of water per kilogram of body weight (9 cc. per pound). He is asked to drink this within the next forty-five minutes. At 9:30, 10:30, and 11:30 A.M., and 12:30 P.M., he is requested to empty his bladder. In order to eliminate the effects of exercise and posture on urinary excretion, he is kept at rest in bed except when up to void. Each specimen is kept in a separate container. The volume of the largest one of these four specimens is measured.

   Under these conditions, some patients having Addison's disease will excrete so little urine that they are unable to void more than one or twice during the entire morning. In such instances, the amount of urine excreted per hour may be calculated; frequently, however, such calculations are unnecessary because of the very low urinary output throughout the entire morning.

Inferences that may be drawn from procedure I:

a. If the volume of any single hourly specimen voided during the morning is greater than the volume of urine voided during the night, the response to the test is negative, that is such a response indicates the absence of Addison's disease.

b. If the volume of the largest hourly specimen voided during the morning is less than the volume of urine voided during the night, the response to the test is positive, that is Addison's disease may or may not be present. To establish the diagnosis, procedure II should be instituted:

Procedure II (based on chemistry of blood and urine): To carry on with this procedure, blood is drawn (preferably under oil) while the patient is still fasting, and the plasma is analyzed for its content of urea and chloride. The specimen of urine that was voided during the night is also analyzed for urea and chloride. From these four determinations and from the results obtained from procedure I, the following equation is solved:

$$A = \frac{\text{Urea in urine (mg. per cent)}}{\text{Urea in plasma (mg. per cent)}} \times \frac{\text{Chloride in plasma (mg. per cent)}}{\text{Chloride in urine (mg. per cent)}} \times \frac{\text{Volume of day urine (cc.)}}{\text{Volume of night urine (cc.)}}$$

The term "day urine" applies to the largest of the hourly specimens voided during the day; "night urine," to the entire amount that was voided from 10:30 P.M. to 7:30 A.M. It is immaterial how these values are expressed, provided that the same method is used throughout the equation. For example, if the concentration of plasma chloride is expressed as milligrams of sodium chloride per 100 cc., the concentration of urinary chloride should be expressed in the same manner.

Inferences that may be drawn from procedure II:

a. If the value of "A" in this equation is greater than 30, the patient probably does not have Addison's disease.

b. If the value for this equation is less than 25, the patient probably has Addison's disease, provided that nephritis has been excluded.

If the results of procedure II are at all equivocal or if they are not indicative of Addison's disease when there is strong clinical evidence to the contrary, the sodium-deprivation test devised by Cutler, Power, and Wilder[11] may be conducted. This can be instituted immediately. When this is done, none of the patient's time is wasted, since the day of the "water test" constitutes the first day of the "provocative test." Cutler and coworkers have encountered only two instances in which it was necessary to resort to their test, and in these cases it also yielded indecisive results.

They also encountered low ratios in cases of nephritis, diabetes insipidus, and in a case of dehydration and fever. With the exception of two cases, the question of Addison's disease did not arise. In these two instances, the sodium-deprivation test excluded Addison's disease. Use of a more complex mathematical expression than the one given has also served to distinguish the response of patients having Addison's disease from those having nephritis.

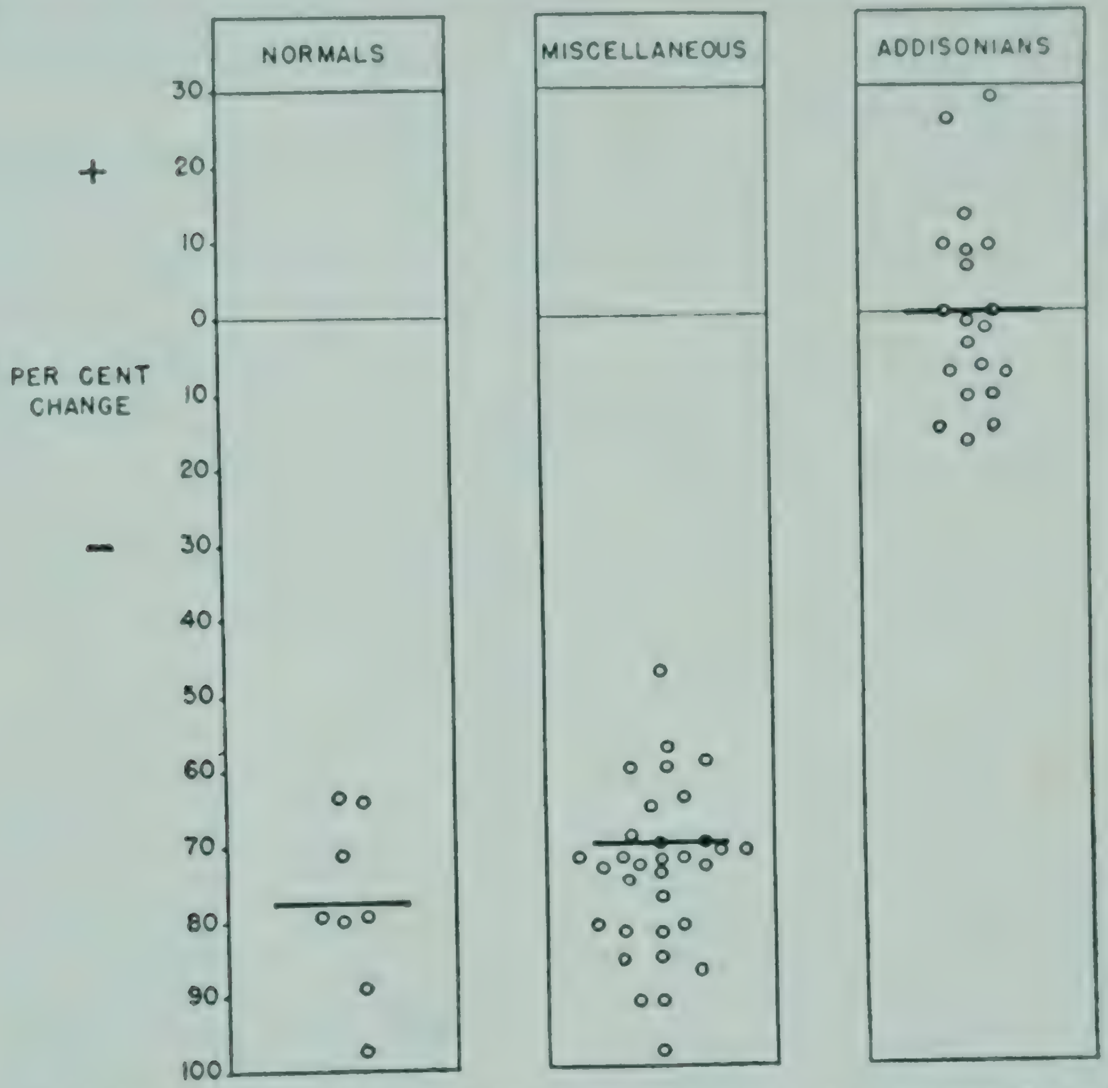

Fig. 2: Hypoadrenalism. Each circle represents percentile change in circulating eosinophils observed in single subject four hours after intramuscular injection of 25 mg. of adrenocorticotropic hormone. Solid bar indicates mean value for group in each instance. (From Thorn, G. W., and others: J.A.M.A., 137:1006, 1948.)

3. Thorn[12] and his associates have worked out a practical test for adrenal cortical insufficiency based upon the response to adrenocorticotropic hormone. In hypoadrenalism, there is little or no fall in eosinophils and little or no increase in the uric-acid–creatinine ratio following the intramuscular injection of 25 mg. of adrenocorticotropic hormone.

The technic is as follows: On the day prior to the test, food is withheld from the patient after 8 P.M. At 6, 8, and 10 A.M. the following day, the patient is given 200 cc. of water by mouth. The control urine consists of the urine secreted from 6 to 8 A.M. The control eosinophil count is done at 8 A.M., following which 25 mg. of adrenocorticotropic hormone (ACTH) is given intramuscularly. The urine secreted from 9 A.M. to 12 NOON is collected, and the eosinophil count is repeated at noon. The uric-acid–creatinine content in each of the two urine specimens is determined by any standard method, and the rise in the uric-acid–creatinine ratio is calculated.

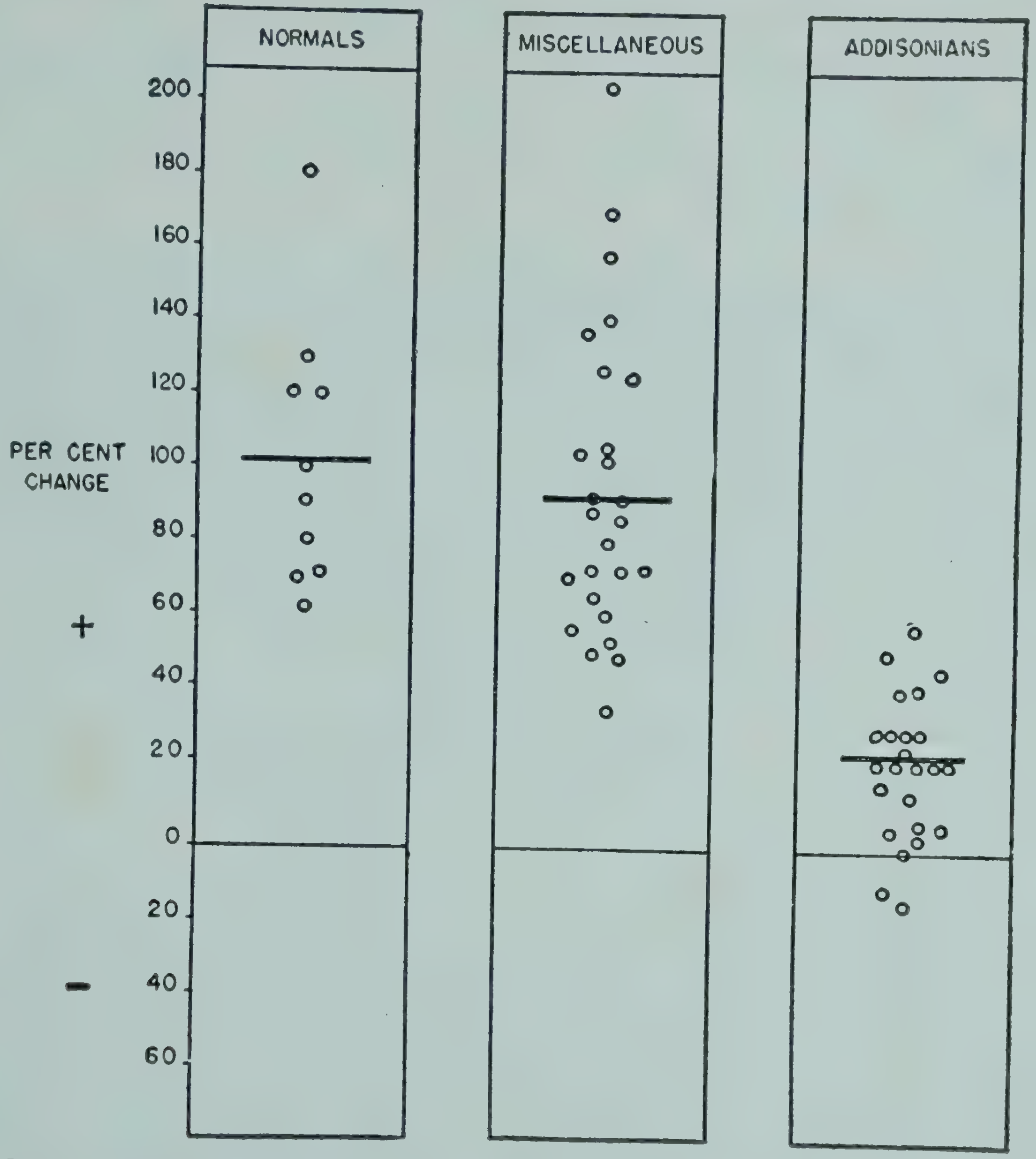

Fig. 3: Hypoadrenalism. Each circle represents percentile change in uric-acid–creatinine ratio observed in single subject four hours after intramuscular injection of 25 mg. of adrenocorticotropic hormone. Solid bar indicates mean value for group in each instance. (From Thorn, G. W., and others: J.A.M.A., 137:1007, 1948.)

A special method is employed for counting the eosinophils in the blood. Five cc. of venous blood is drawn into a small collecting bottle containing the dried residue of 0.5 cc. of a balanced oxalate solution (1.2 Gm. ammonium oxalate and 0.8 Gm. potassium oxalate made up to 100 cc. with distilled water). Oxalated blood is drawn into a white-cell-count pipet up to the 0.5 mark. The pipet is then filled with a special diluting fluid and shaken for thirty seconds. The counting chamber is filled, and the eosinophils are counted. Eosinophils appear bright red against a pink background. The other leukocytes are colorless. The diluting fluid that is used consists of 5 cc. of 2 per cent eosin solution in water, 5 cc. of acetone, and distilled water sufficient to make 100 cc. Because of its large capacity, Thorn uses the Levy counting chamber, which has a depth of 0.2 mm. and a ruled area of 16 sq. mm. It is important to shake the diluting pipet for not more than thirty seconds, or some of the eosinophils may disintegrate. Furthermore, the count should be made promptly after dilution has been accomplished. The average count of four 16 sq. mm. squares multiplied by 6.25 gives the number of eosinophils per cubic millimeter. The decrease in eosinophils is expressed as the percentage of the initial count. The fall in eosinophils and rise in uric-acid excretion reach their maxima in about four hours. A reduction of 50 per cent or more in the uric-acid–creatinine ratio during the test is presumptive evidence that the cortical tissue has normal capacity to respond to stimulation.

A more practical modification of the foregoing technic is now in general use. A basal eosinophil count is made in the morning with the patient in a fasting state. Immediately after the blood is drawn for the eosinophil count, the patient is given 0.3 cc. of a 1:1000 epinephrine solution subcutaneously. The patient may then eat breakfast. A second eosinophil count is made four hours after the epinephrine injection, after which lunch may be served. The percentage of fall of eosinophils is interpreted in the same way as after giving adrenocorticotropic hormone.

4. Serum sodium is usually decreased, and serum potassium is increased.
5. The fasting blood glucose is reduced, and the patient has increased tolerance for glucose.
6. The basal metabolic rate is decreased.
7. Nonprotein nitrogen is usually increased because of kidney failure.
8. Total proteins of the plasma are elevated because of dehydration.
9. The sodium-deprivation test, introduced by Cutler, Power and Wilder,[11] is rarely used because of the danger to the patient.

10. The plasma chloride is reduced, and there is an increase in the urine chloride.
11. There is usually a moderate normocytic or microcytic anemia.
12. The urine may contain albumin and casts.

THYROID

HYPERFUNCTION

Hyperthyroidism:

This condition is due to secretion of excessive amounts of thyroxin by a hyperplastic thyroid gland. The most common form of hyperthyroidism is exophthalmic goiter, or Graves' disease. It is characterized by goiter, exophthalmos, tachycardia, tremor, and increased basal metabolic rate. Other symptoms and signs that serve to complete the picture are purposeless movements, irritability, increased sweating, increased tendon reflexes, loss of weight, dyspnea, palpitation, psychoses, and sensitivity to heat.

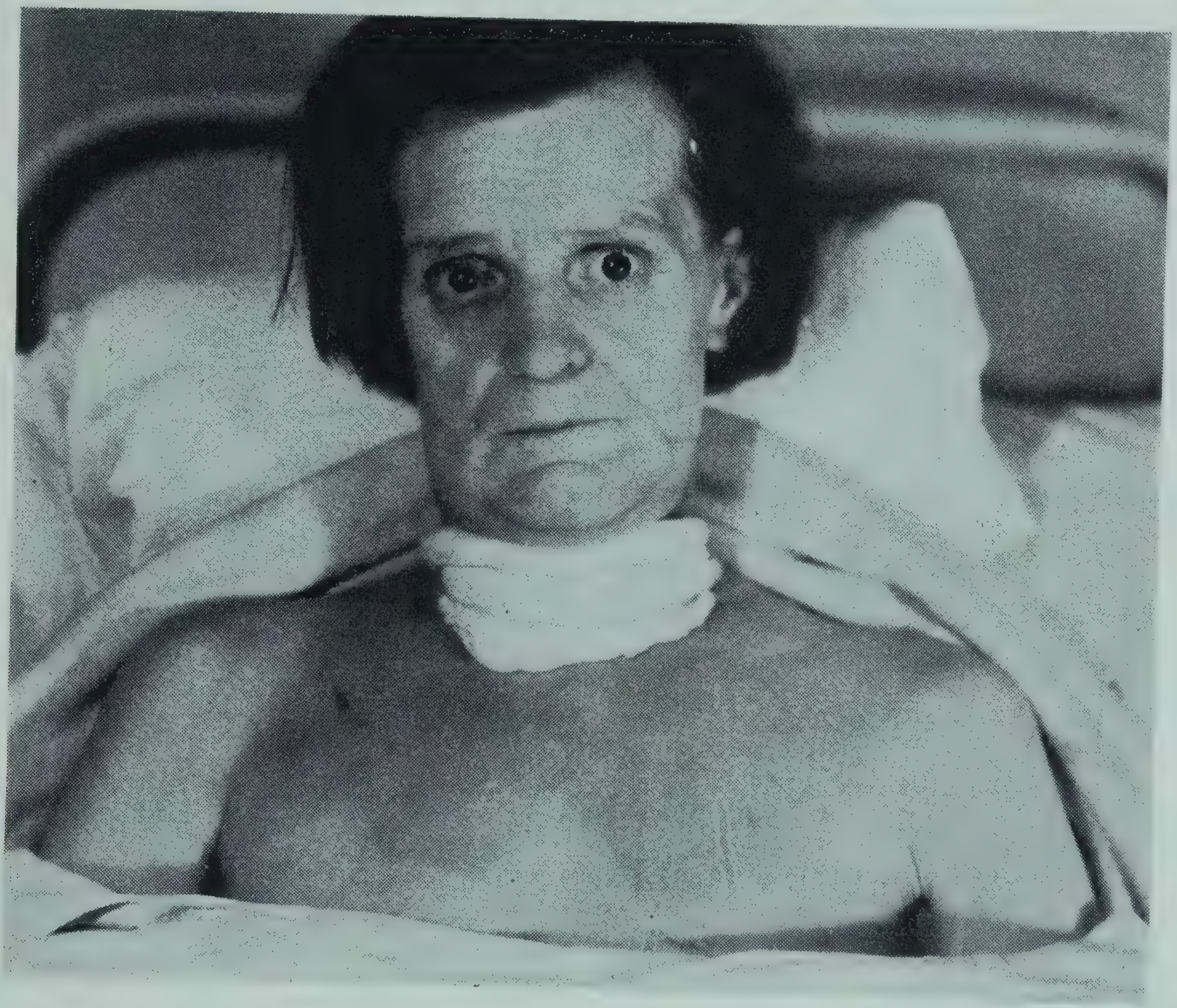

Fig. 4: Patient with hyperthyroidism.

Laboratory Examinations:

1. The basal metabolic rate is usually increased and may be elevated to +100.
2. The blood-iodine level is increased, but this test is rarely used.
3. An increased utilization of iodine is noted in the iodine-tolerance test because the tissues are undersaturated in hyperthyroidism.
4. A decrease in blood cholesterol is significant if present. The total lipids are also usually decreased.[13,14]
5. Hyperglycemia is present, and there is decreased tolerance to glucose.
6. The liver is frequently damaged; and as a result, the galactose-tolerance test and the hippuric-acid test show evidence of functional impairment. If further investigation of the liver is indicated, see Chapter 23 (p. 432).
7. Glycosuria is frequently present.
8. Excretion of calcium and phosphorus in the urine is increased according to Aub and associates,[15] but these examinations are not required to make a diagnosis.
9. Creatinuria has been reported by Shaffer.[16]

HYPOFUNCTION

Hypothyroidism:

A deficient secretion of the thyroid hormone brings about a variety of clinical syndromes depending upon the age of the person and the degree of the deficiency. The syndromes may be grouped as follows:

1. Cretinism, a hypothyroid condition that occurs in infancy
2. Juvenile hypothyroidism, a condition that is milder than cretinism and occurs in childhood
3. Hypothyroidism of adult life, which is of varying degrees of severity
4. Myxedema, a state of extreme hypothyroidism occurring in adults

These patients have diversified symptoms, but many have increased sensitivity to cold, fatigability, skin rashes, vague abdominal and joint pains, weight changes, retardation of bone development and enuresis (in children), menstrual disorders, and sterility (in men).

These conditions, representing various degrees of hypothyroidism, produce similar laboratory findings.

Laboratory Examinations:

1. The basal metabolic rate is decreased, and values as low as —45 are obtained in the more severe cases.
2. Blood cholesterol is constantly increased.[17]

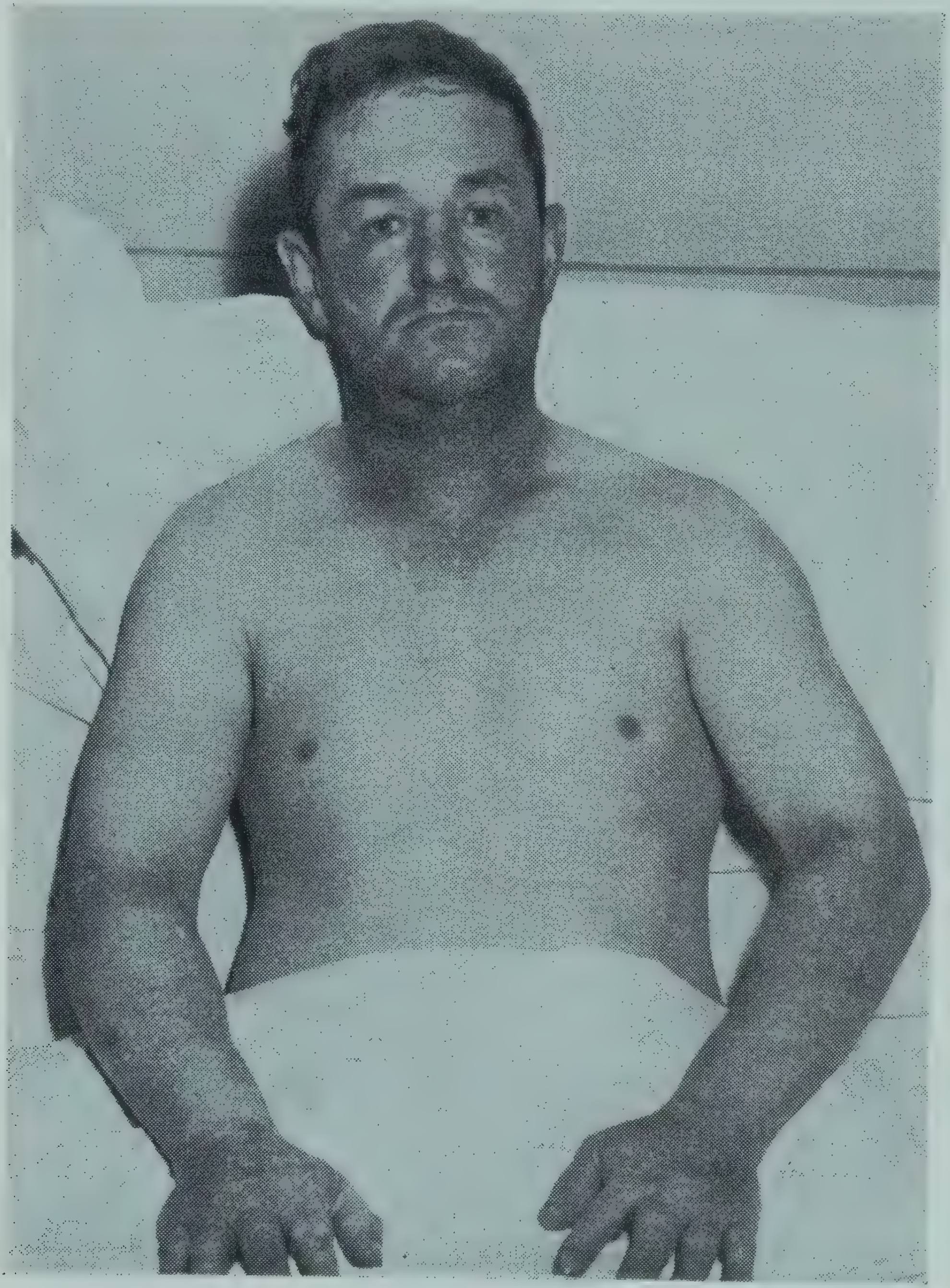

Fig. 5: Patient with hypothyroidism. Note coarse skin of forearms and hands.

3. The fasting blood glucose is usually decreased, and there is an increase in tolerance to glucose.
4. The blood-iodine level is decreased.
5. According to Elmer,[18] there is a decreased utilization of iodine in the iodine-tolerance test because the thyroid gland and other body tissues are unable to take up iodine from the blood as well as normally.
6. There is a moderate degree of anemia.

PARATHYROID

HYPERFUNCTION

Hyperparathyroidism:

This condition results from overactivity of the parathyroid gland with excessive secretion of parahormone. The increased activity is due to hyperplasia or adenoma of one or more parathyroid glands. The results are disturbances of the calcium and phosphorus metabolism.

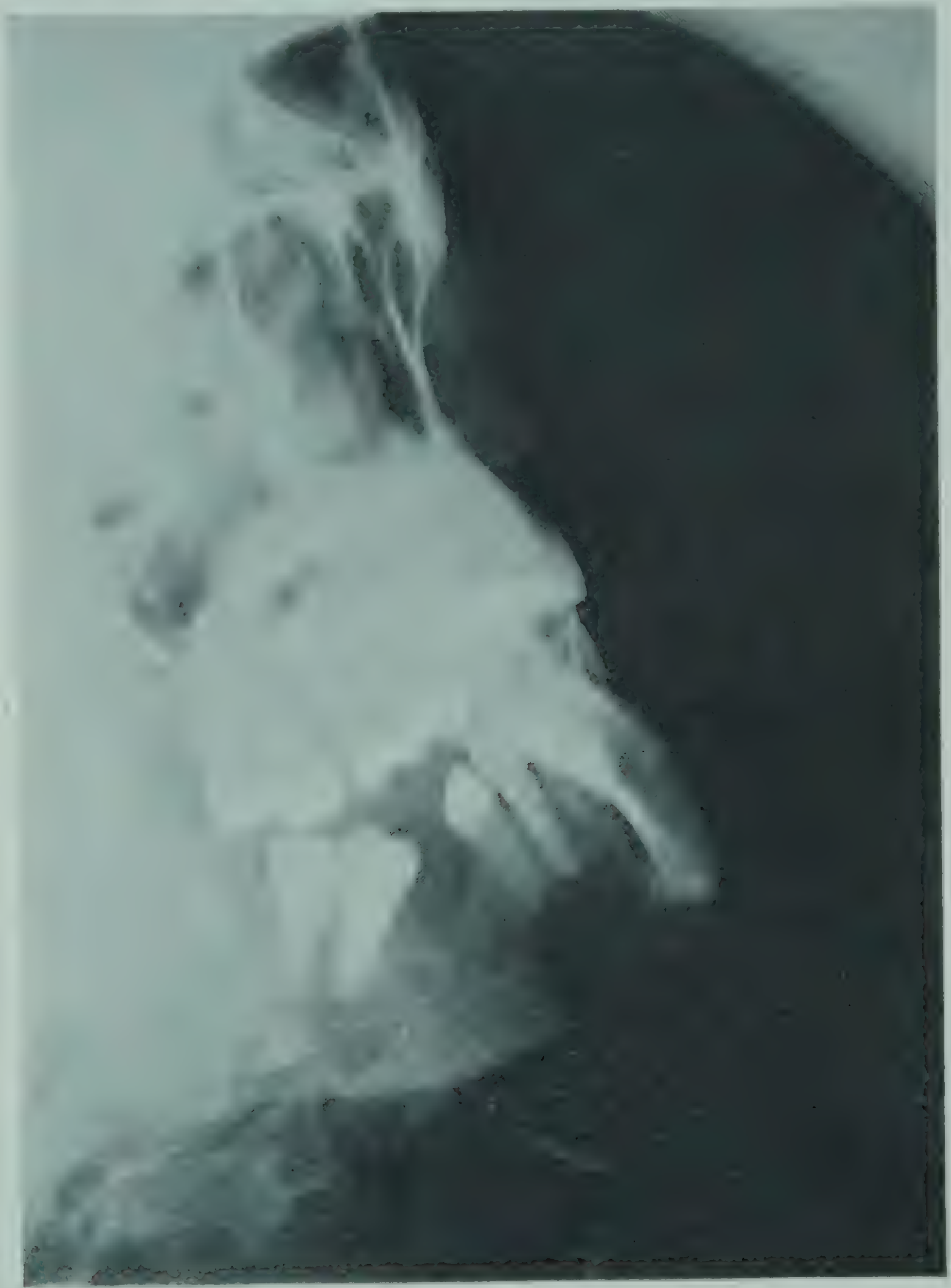

Fig. 6: Osteitis fibrosa cystica of anterior part of mandible. This patient had increased blood calcium, decreased phosphorus, and increased alkaline phosphatase.

The hypercalcemia causes symptoms of weakness, loss of weight, anorexia, muscle, joint, and abdominal pains, incoordination, cardiac irregularities, neurasthenia, polyuria, polydipsia, and renal pain.

The excessive excretion of calcium and phosphate in the urine causes polyuria, polydipsia, nephrolithiasis, dysuria, albuminuria, and hematuria.

The prolonged withdrawal of calcium from the bones results in decalcification, bone cysts, fractures, and deformities. The disease, which is known as generalized osteitis fibrosa cystica, or von Recklinghausen's disease, is now believed to be a manifestation of a late stage of hyperparathyroidism.

Laboratory Examinations:

1. The blood calcium is increased above 12 mg. per 100 cc. It is advisable to do repeated examinations sometimes to establish an elevation of the calcium.
2. The alkaline phosphatase is increased.
3. The blood-serum, inorganic phosphate is decreased.
4. Calcium and phosphate excretion in the urine are markedly increased. For comparison, the calcium excretion should be determined with the patient on a diet free of calcium.

Multiple Myeloma:

The parathyroid glands are enlarged in multiple myeloma, but there is apparently no etiological relationship between the two.

Laboratory Examinations: See p. 381.

Osteitis Deformans:

Secondary changes may occur in the parathyroid glands, but the exact cause of this disorder is not known. The process is a rarefying osteitis involving the centers of the bones with new bone formation in the subperiosteal regions.

Laboratory Examinations:

1. The serum alkaline phosphatase is increased.
2. The blood calcium and phosphorus are normal.

HYPOFUNCTION

Hypoparathyroidism:

This may result from any cause that brings about deficient production of hormone. The most common causes of hypoparathyroidism are accidental injury or removal in the course of operation on the thyroid, or fibrosis, sclerosis, or hemorrhage.

The symptoms are due chiefly to hypocalcemia, which causes tetany. Tingling sensations in the hands and feet are noted first. Later, carpopedal spasm, laryngospasm, and convulsions of tetany become evident.

Tetany may occur in infants as the result of alkalosis, hyperventilation, or loss of hydrochloric acid from the stomach rather than from deficient function of the parathyroid glands. Certain cases of tetany are difficult to explain on the basis of calcium deficiency, since both diffusible and nondiffusible calcium levels are normal.

Laboratory Examinations:

1. The serum calcium is reduced, particularly its diffusible fraction. It is reduced frequently to 5 or 6 mg. per 100 cc.
2. The inorganic phosphate is increased and ranges from 4 to 6 mg. per 100 cc.
3. The magnesium level of the blood is reduced and ranges from 1.5 to 1.8 mg. per 100 cc.
4. The urinary excretion of calcium and inorganic phosphate is decreased in tetany.

ISLANDS OF LANGERHANS

Hyperfunction

Hyperinsulinism:

The islands of Langerhans function as an endocrine gland within the pancreas; and they produce insulin, which is necessary to the proper metabolism of sugar. These islands may undergo hyperplasia or tumor formation and thus secrete an excessive amount of insulin. The excess of insulin in the bloodstream causes hypoglycemia with associated nervousness, weakness, trembling, sweating, emotional instability, inability to concentrate, and drowsiness.

Laboratory Examinations:

1. The fasting blood glucose is reduced and ranges from 60 to as low as 30 mg. per 100 cc.
2. The patient shows increased tolerance to glucose as evidenced by a rise, usually not exceeding 120 mg. per 100 cc., and returning to subnormal again within two hours.

HYPOFUNCTION

Diabetes Mellitus:

This condition represents hypofunction of the islands of Langerhans with disturbance of sugar metabolism, which is the opposite of that caused by hyperinsulinism. The result of this deficiency of insulin is hyperglycemia, with or without glycosuria, and, in severe cases, with acidosis.

Diabetes mellitus is characterized by polyuria, polydipsia, polyphagia, loss of strength, loss of weight, pruritis, dry skin and mouth, and pain in the back and extremities. In children, the symptoms are loss of weight, lassitude, easy fatigability, vomiting, abdominal pain, and enuresis.

Laboratory Examinations:

1. Glucose is present in the urine. Other sugars, such as pentose, lactose, levulose, and galactose, and other reducing substances, such as glycuronic acid, homogentisic acid of alkaptonuria, creatinine, uric acid, nucleoprotein, and conjugate glycuronates, should be differentiated from glucose.

   Renal glycosuria and nondiabetic transient glycosuria should also be differentiated from true diabetic glycosuria.
2. The urine should be examined for the ketone bodies: acetone, diacetic acid, and betahydroxybutyric acid. The presence of ketonuria is evidence of acidosis, which may be more accurately investigated by doing a carbon-dioxide combining power on the blood.
3. The determination of the fasting, blood-glucose level is of great importance in making a diagnosis and in following the therapy. The fasting glucose level may be normal in mild diabetic patients, and the presence of the disease may be detected only by a glucose-tolerance test. In diabetes, the fasting glucose may reach 600 mg. per 100 cc. or more.

   Glucose determinations of the blood are of value as a guide in adjusting the dosage of insulin. A glucose determination should also be done in diabetic coma in order to guide insulin therapy.
4. Glucose-tolerance tests are valuable in determining latent diabetes and in surveying a diabetic family for the detection of diabetes. The continuance of the test should be guided by the fasting glucose level. It is not wise to give glucose to a diabetic patient with a level of 200 mg. or more per 100 cc. In fact, a glucose-tolerance test is contraindicated in known cases of diabetes. It

is of most value in borderline cases where the fasting blood glucose is 140 to 160 mg. per 100 cc.

5. The determination of the carbon-dioxide combining power is important in cases of coma or evident acidosis.
6. The blood cholesterol is increased in severe diabetes and in diabetic coma, and is of particular value in children in whom diabetes is very difficult to control.
7. The total blood lipids in severe diabetes and in coma are increased from a normal of 0.4 to 0.6 Gm. to 2.5 Gm. or more per 100 cc.
8. The blood chlorides are reduced because of vomiting or excessive excretion in the urine, or possibly because of transfer of plasma chloride to the red cells. The hypochloremia is associated with oliguria or anuria.
9. The nonprotein nitrogen may be increased in diabetes in conditions other than nephritis, such as hypochloremia, dehydration, shock, or excessive breakdown of protein.
10. The blood potassium level is reduced in diabetic coma to 2 mEq. per liter or even lower (normal 4.1 to 5.6 mEq. per liter). Associated with this decrease in potassium is a change in respiration to the rapid, shallow Kussmaul type. This reduction in potassium may be due to (1) effect of insulin therapy, or (2) diuresis and dehydration with resultant loss of water and potassium. The effect of insulin injection is confirmed by the report of Harrop and Benedict,[19] who found that an injection of insulin in patients with diabetes mellitus produced a reduction in serum potassium. In diuresis and dehydration not due to diabetic acidosis, Elkinton and Winkler[20] confirmed the loss of potassium in the urine.

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CHAPTER 26

Central Nervous System

Valuable information is obtained from the laboratory examination of the fresh cerebrospinal fluid in diseases of the central nervous system. In fact, bacteriological and serological examinations frequently reveal the diagnosis, while cytologic and chemical examinations give confirmatory or prognostic information.

BACTERIAL INFECTIONS

Purulent Meningitis:

Meningitis supervenes when any of the pathogenic bacteria gain access to the meninges and cerebrospinal fluid. Meningitis is caused by meningococci, pneumococci, streptococci, Hemophilus influenzae, staphylococci, gonococci Pseudomonas aeruginosa, Escherichia coli, Corynebacterium diphtheriae, Neisseria catarrhalis, Klebsiella pneumoniae, B. anthracis, Eberthella typhosa, and Salmonella paratyphi. Most of the organisms mentioned above cause similar changes in the cerebrospinal fluid, thus they will be discussed as a group from the point of view of laboratory examinations.

Laboratory Examinations:

1. Bacteriological examination by smear and culture is of paramount importance in establishing the etiological agent. It is important

that the clinician be notified as soon as the type of organism has been established in the smear. It is also important to notify the clinician if no organism is found in a smear made up of leukocytes because, in such a case of meningitis, the causative organism is frequently the meningococcus. The cultures usually reveal the organism and its type.

2. The white-cell count of the cerebrospinal fluid may range from 100 to 10,000 or more per cubic millimeter. Cell counts done early in the disease are likely to be lower. The cells are 90 to 100 per cent polymorphonuclear leukocytes. As the patient recovers, the cell count decreases, and lymphocytes and monocytes are seen in increasing numbers.
3. The leukocytes give the fluid a "ground-glass" appearance early in the disease. It may become cloudy or purulent later in the disease. A coagulum usually forms, leaving a fairly clear, slightly yellow, supernatant fluid.
4. Quantitative protein determination of the cerebrospinal fluid shows variable elevations, ranging from 100 to 1000 mg. per 100 cc. Qualitative protein tests are positive.
5. The glucose level of the fluid is decreased in purulent meningitis and ranges from 50 to 10 mg. per 100 cc. In some cases, it may become too low to read. The decreased glucose level is apparently due to the utilization of this agent by the bacteria. As the disease progresses to a fatal termination, the glucose level becomes lower, but it increases as the patient improves following treatment with sulfonamides or antibiotics.

   The cerebrospinal-fluid glucose should be correlated with the blood-glucose level because the two parallel each other. A low, normal, cerebrospinal-fluid, glucose level is evidence of a reduction in a person with a high blood glucose.
6. The chloride level is reduced in meningitis, but the reduction is less marked in purulent meningitis than in tuberculous meningitis.
7. In the colloidal-gold test, most cases show a meningitic curve, but curves in zones I and II are noted in the same patients.
8. A blood culture may reveal the same organism that is present in the cerebrospinal fluid. This is particularly true in meningitis caused by the meningococcus, pneumococcus, and other organisms that have reached the meninges by way of the bloodstream.
9. There is an increase in the white-cell count of the blood associated with purulent meningitis, except in diseases that have a leukopenia, such as typhoid fever.

TABLE 1: SUMMARY OF USUAL CEREBROSPINAL-FLUID CHANGES IN DISEASE

Disease	*Pressure*	*Cell Count*	*Protein Content*	*Sugar Content*	*Chloride Content*	*Colloida Gold*
No disease (normal) . .	80 to 180 mm.	0 to 5	15 to 45 mg.	50 to 80 mg.	700 to 750 mg.	0000000
Acute purulent meningitis.	+ + +	+ + +	+ + +	− − −	− −	2 or 3
Tuberculous meningitis.	+ +	+ or + +	+ or + +	− or − −	− − or − − −	2 or 1
Benign lymphocytic meningitis.	N or +	+ or + +	N or +	N	N or −	N or 2
Acute anterior poliomyelitis.	N or +	+ to + +	N or + +	N	N or −	N or 2
Epidemic encephalitis. .	N	N to + +	N or +	N	N	N or 2
Aseptic meningeal reaction.	+ to + + +	+ to + + +	N to + +	N	N or −	2 or 1
Dementia paralytica. . .	N	+ to + +	+ to + +	N or −	N or −	1 or 2
Tabes dorsalis.	N	+ to + +	+ to + +	N	N	2 or 1
Meningeal neurosyphilis.	+ to + + +	+ to + + +	+ to + +	N or −	N or −	2, 1, or
Vascular neurosyphilis.	N to +	N to + +	N to + +	N	N or −	N, 2, or
Multiple sclerosis.	N	N or +	N or +	N	N	N, 1, or
Acute encephalomyelitis and acute transverse myelitis.	N or +	+ or + +	N or +	N	N	N, 1, or
Brain tumor.	+ to + + +	N or +	+ to + +	N	N or −	N, 2, or
Brain abscess.	+ to + + +	+ to + + +	N to + +	N	N or −	N, 2, or
Spinal-cord tumor. . . .	N	N or +	+ to + + +	N	N or −	N, 2, 3,
Epilepsy.	N	N	N	N	N	N
Polyneuritis.	N	N or +	N to + + +	N	N or −	N, 2, or
Subdural hematoma. . .	+ to + + +	N to +	N to + +	N	N	N or 2
Cerebral thrombosis. . .	N to +	N or +	N or +	N	N	N or 2
Cerebral hemorrhage. .	+ to + + +	+	+ to + + +	N	N or −	N, 2, 3,
Subarachnoid hemorrhage.	+ to + + +	+	+ to + + +	N	N or −	N, 2, 3,

Explanation of Symbols:
N = Normal + = Slightly increased + + = Moderately increased + + + = Greatly increa
− = Slightly decreased − − = Moderately decreased − − − = Greatly decrea
1 = First-zone (5555432100) 2 = Mid-zone (0123332100) 3 = End-zone (0011233

TABLE 1: SUMMARY OF USUAL CEREBROSPINAL-FLUID CHANGES IN DISEASE*

Comment
Fluids purulent and xanthochromic; coarse clot; subarachnoid block may occur; organisms present on smear and culture
Fluids clear to ground-glass; xanthochromic; fine clot; subarachnoid block may occur; organisms on smear and by guinea-pig inoculation
Fluids clear or ground-glass; differentiated from tuberculous and purulent meningitis by normal sugar, from syphilis by negative Wassermann
Pressure increased in high cervical or bulbar cases with respiratory difficulty; cells decrease rapidly; protein may increase
Cells, 100 per cent lymphocytes; differentiated from brain tumor and abscess by normal pressure, from tuberculous meningitis by normal sugar, and from syphilis by negative Wassermann
Fluids clear to cloudy; sterile culture and normal sugar exclude purulent meningitis; negative Wassermann excludes syphilis
Fluids always abnormal in untreated cases; Wassermann positive in 0.1 to 0.05 cc. of fluid
Wassermann positive in 0.1 to 1 cc. of fluid; late "burnt-out" or treated cases may have normal fluid
Fluids clear to ground-glass; xanthochromic; fine clot; differentiated from tuberculous meningitis by Wassermann test and normal chlorides
Fluids clear or xanthochromic; Wassermann reaction positive in fluid or serum
Normal pressure differentiates from brain tumor, normal dynamics from spinal-cord tumor, negative Wassermann from syphilis
Fluids clear or xanthochromic; pressure normal or only slightly elevated; in spinal cases, cord tumor or epidural abscess excluded by normal dynamics
Pressure and protein increased except in a few cases; fluids frequently xanthochromic; syphilis excluded by negative Wassermann
Pressure practically always increased; fluids clear to cloudy and xanthochromic with clot; polymorphonuclear leukocytes usually present; meningitis excluded by normal sugar and negative culture
Dynamic block practically always present; fluids xanthochromic with high protein
Brain tumor excluded by normal pressure; syphilis, by negative Wassermann
Fluids usually normal except for slight increase in protein in alcoholic patient, metal poisoning, or vitamin-deficiency neuritis; very high protein, pleocytosis, and abnormal gold curve in so-called "polyneuritis with facial diplegia"
Fluids bloody or xanthochromic in early cases; clear and colorless in late cases; pressure occasionally normal
Pressure usually normal; fluids clear or slightly xanthochromic; pressure greater than 300 mm. or protein greater than 100 mg. suggests brain tumor; negative Wassermann excludes syphilis
Fluids usually bloody and xanthochromic; red cells gradually disappear; fluid becoming more xanthochromic; subarachnoid differentiated from cerebral hemorrhage by absence of focal signs

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*From the Cyclopedia of Medicine, Surgery and Specialties. F. A. Davis Company, Philadelphia, 1948, p. 148.

Tuberculous Meningitis:

This type of meningitis is secondary to a tuberculous focus elsewhere in the body. The disease has always ended fatally, but with the advent of streptomycin the outlook is somewhat more encouraging. It is important to establish the fact that the strain of Mycobacterium tuberculosis in a given case is sensitive to streptomycin.

Laboratory Examinations:

1. Bacteriological examination with smear, culture, and guinea-pig inoculation is important in establishing the diagnosis. The organism is more likely to be found in the pellicle.
2. The cell count is increased to 500 or more per cubic millimeter with lymphocytes predominating.
3. The fluid is either clear or turbid and is characterized by the formation of a delicate pellicle. The supernatant fluid is frequently slightly yellow.
4. Quantitative protein determination of the fluid shows an increase ranging from 50 to 600 mg. or more per 100 cc. Qualitative protein tests are positive.
5. The glucose level decreases progressively until it is too low to read.
6. The chloride level is characteristically reduced and is usually below 650 mg. per 100 cc. It decreases progressively as the disease advances.
7. The colloidal-gold test may be normal or show a Zone-I, -II, or -III curve.
8. The Levinson test is positive. See p. 181.

Syphilitic Meningitis:

This type of meningitis usually occurs during the secondary stage of syphilis.

Laboratory Examinations: A serological test for syphilis is usually positive in both the blood and cerebrospinal fluid.

FINDINGS IN CEREBROSPINAL FLUID:

1. The spirochetes have been found by darkfield examination of the cerebrospinal fluid, but this is not a usual procedure.
2. The cell count varies from 10 to 1000 or more, and lymphocytes predominate.
3. The fluid varies from colorless to light yellow. A coagulum forms in most cases.

4. Quantitative protein determination of the fluid shows an increase ranging from 50 to 300 mg. per 100 cc. or more. The qualitative tests for protein are almost always positive.
5. The glucose level is normal or moderately decreased, ranging from 85 to 30 mg. per 100 cc., and occasionally it may be lower.
6. The chloride level is normal or slightly decreased and ranges from 745 to 640 mg. per 100 cc.
7. The colloidal-gold reaction shows either a Zone-I or Zone-II reaction.

Syphilis of Central Nervous System:

The important syphilitic diseases of the central nervous system are syphilitic vascular disease, tabes dorsalis, and dementia paralytica. The laboratory findings may be summarized as in Table 2.

TABLE 2: LABORATORY FINDINGS IN SYPHILIS OF CENTRAL NERVOUS SYSTEM

Test	*Syphilitic Vascular Disease*	*Tabes Dorsalis*	*Dementia Paralytica*
Blood test for syphilis	Positive in 80 per cent of cases	Usually positive	Usually positive
Cerebrospinal fluid test for syphilis	Positive in 57 per cent of cases	Usually positive	Positive
Cell count	10 to 100 per cubic millimeter; Lymphocytes predominate	10 to 75 per cubic millimeter; Lymphocytes predominate	25 to 200 per cubic millimeter; Lymphocytes predominate
Protein	30 to 150 mg. per 100 cc.	30 to 100 mg. per 100 cc.	50 to 100 mg. per 100 cc.
Globulin	Usually positive	Doubtful to positive	Positive
Colloidal gold	Positive usually in Zone II	Positive usually in Zone II	Positive in Zone I

Brain Abscess:

The causative organism is not usually found in the cerebrospinal fluid unless the abscess impinges upon the meninges and actually opens into the cerebrospinal fluid. The laboratory findings in the cerebrospinal fluid are consequently not diagnostic.

Laboratory Examinations:

1. The cell count may vary from 0 to 1000 or more depending upon the relationship of the abscess to the meninges. Polymorphonuclear leukocytes predominate. As the abscess becomes encapsulated, the cell count decreases and lymphocytes increase.
2. Quantitative protein is increased and ranges from 45 to 200 mg. per 100 cc.
3. The glucose level is normal or slightly increased. It may range from 60 to 90 mg. per 100 cc. but is decreased if meningitis develops.
4. The chloride level is normal or slightly decreased; but in the presence of meningitis or pneumonia, the level is markedly decreased.

Aseptic Reaction to Septic Foci:

Subdural abscess, septic embolus, and mastoid infection cause a sterile inflammatory reaction in the cerebrospinal fluid.

Laboratory Examinations:

1. The cerebrospinal-fluid cell count is variable with a range of from 5 to 1000 or more. Polymorphonuclear leukocytes predominate.
2. The fluid is clear to cloudy depending upon the number of cells.
3. The glucose level is normal or slightly decreased.
4. The chloride level is normal or only slightly decreased.
5. The fluid is usually sterile, but occasionally the bacteria from the infectious process gain access to the meninges. When this occurs, the changes in the fluid are those of meningitis.

MYCOTIC INFECTIONS

The following fungi and yeasts have been reported as causing meningitis in human beings: Cryptococcus neoformans, Actinomycetes, Coccidioides immitis, Sporotricha, Blastomyces dermatitidis, Aspergilli, Candidae, and Leptothrices. The diagnosis is made by isolating the fungus from the cerebrospinal fluid.

Cryptococcosis Encephalitis:

Sometimes, yeasts gain access to the cerebrospinal fluid and cause a chronic meningitis. These are chiefly of the Cryptococcus group.

Laboratory Examinations:

1. The round yeast bodies are found in the counting chamber or in the stained smear. In the counting chamber, they may be confused with lymphocytes or even erythrocytes. However, the characteristic buds of yeasts should identify them. They may be cultured on Sabouraud's media.
2. The cell count is increased, and lymphocytes usually predominate. However, this is not constant and polymorphonuclear cells sometimes predominate.
3. The protein level is increased.
4. The glucose level is decreased.
5. The chloride level is decreased.

PARASITIC INFECTIONS

Trichinosis:

The larvae of Trichinella spiralis are sometimes found in the cerebrospinal fluid.

Laboratory Examinations:

1. The identification of the larvae is diagnostic, but apparently they are only in the cerebrospinal fluid temporarily.
2. The cell count may be increased.
3. Protein may be increased. However, the fluid may be quite normal in the presence of the parasite.
4. The intradermal test and precipitin test should be carried out to confirm the diagnosis if the parasites are not found in the fluid.

Trypanosomiasis:

This is African sleeping sickness, which is caused by Trypanosoma gambiens or Trypanosoma rhodesiense. Sometimes the parasites are found in the cerebrospinal fluid.

Laboratory Examinations:

1. The identification of the trypanosomes in the cerebrospinal fluid is diagnostic.
2. The cell count is variable and ranges from normal to as high as 2000 per cubic millimeter. Lymphocytes predominate, although plasma cells and monocytes may be present.
3. The protein level is increased up to 100 mg. per 100 cc.
4. The glucose level is normal, according to Broden and Rodhain.[1]

VIRAL INFECTIONS

Anterior Poliomyelitis:

This disease involves chiefly the spinal cord, and it produces certain changes in the cerebrospinal fluid that are confirmatory rather than diagnostic. Identification of the virus is not at present a clinical laboratory procedure. The following tests are carried out on the cerebrospinal fluid:

Laboratory Examinations:

1. The cell count is variable, but in the majority of cases it is less than 300 per cubic millimeter. In the first few days, polymorphonuclear leukocytes predominate; later, lymphocytes predominate. The cell count decreases after the onset of paralysis and is normal in three weeks.
2. The fluid may be clear or turbid. A delicate pellicle may form on standing.
3. The protein level may be normal in the preparalytic stage. It then rises to 300 mg. or more. Both albumin and globulin are increased.
4. The glucose level is normal.
5. The chloride level is usually normal.
6. The colloidal-gold curve when positive shows a Zone-II curve.

Epidemic Encephalitis:

Variations from normal in the cerebrospinal fluid are slight and are not diagnostic.

Laboratory Examinations:

1. The cell count varies from normal to 100 per cubic millimeter and only rarely reaches a higher figure. The count tends to rise in the first few weeks. Lymphocytes predominate, and a few plasma cells and monocytes are present. Polymorphonuclear leukocytes are rarely found.
2. The fluid is clear, and a pellicle does not form.
3. The protein level is usually within normal limits, but occasionally values up to 100 mg. per 100 cc. are found.
4. The glucose level is normal or slightly increased. An increase in the glucose level is not now considered diagnostically significant. It is chiefly of value in differentiating encephalitis from tuberculous meningitis.
5. The chloride level of the fluid is usually within normal range.

6. The colloidal-gold reaction shows a Zone-II curve in about half of the cases. In the other half, the curve is normal.

Postvaccinial and Postinfectious Encephalitis:

Encephalitis or encephalomyelitis sometimes occurs after smallpox vaccination and after certain infections, such as erysipelas, scarlet fever, chorea, pertussis, pneumonia, typhoid fever, typhus fever, streptococcus septicemia, and so on.

Laboratory Examinations:

1. The cell count is normal or slightly increased, and lymphocytes predominate. The fluid is clear.
2. The protein level is normal or only slightly increased, sometimes reaching 100 mg. per 100 cc.
3. The glucose level is normal or slightly increased.
4. The chlorides are normal or slightly decreased.
5. The colloidal-gold curve is usually normal.

A comparison of the cerebrospinal-fluid findings in the encephalitides is shown in Table 3.

Benign Lymphocytic Meningitis:

This is also designated as "virus meningitis," "lymphocytic choriomeningitis," and "acute lymphocytic meningitis." There are probably other causes than a single virus.

Laboratory Examinations:

1. Demonstration of the virus by animal inoculation with the cerebrospinal fluid is ideal but not practical for the average clinical laboratory. The demonstration of virus neutralizing antibodies likewise is not carried out in the average laboratory. Bacteriological examination reveals a sterile fluid.
2. The cell count is increased and may reach as high as 3000 per cubic millimeter. They are practically all lymphocytes.
3. The protein level is normal or only slightly increased.
4. The glucose level is normal.
5. The chloride level is within normal range.
6. The colloidal-gold reaction may show a meningitic curve.

Meningitis of Mumps:

The meninges are involved in an appreciable number of cases of mumps. The cerebrospinal fluid reflects the invasion of the meninges in these cases.

TABLE 3: THE CEREBROSPINAL FLUID IN ENCEPHALITIS

Type of Encephalitis	*Pressure*	*Color*	*Appearance*	*Pellicle*	*Cell Count*	*Type of Cells*	*Qualitative Protein*	*Quantitative Protein*	*Glucose*	*Chloride*	*Colloidal Gold*	*Virus Neutralization Tests*
Lethargic	Normal or slightly increased	Colorless or slightly xanthochromic	Clear	Rare	10 to 200	Lymphocytes	Doubtful or positive	Slight increase	Normal	Normal	Usually positive	None
Equine	Normal or slightly increased	Colorless	Clear or ground glass	Rare	200 to 2000	Lymphocytes	Weak to strongly positive	Increase	Normal	Normal	Usually positive	Positive
St. Louis	Normal or slightly increased	Colorless	Clear or ground glass	Rare	50 to 1000	Lymphocytes	Negative or weakly positive	Usually increased	Usually increased	Normal or slight increase	Usually positive	Positive
Japanese	Normal or slightly increased	Colorless	Clear	None	10 to 100	Lymphocytes	Doubtful to weakly positive	Slight increase	Normal	Normal	Usually positive	Positive
Australian X Disease	Normal or slightly increased	Colorless	Clear	None	10 to 100	Lymphocytes	Weakly positive to moderately positive	Slight increase	Normal	Normal	Usually positive	Positive
Post-infectious	Increased	Colorless	Clear	None	10 to 100	Lymphocytes and monocytes	Doubtful to weakly positive	Slight increase	Normal or slight decrease	Normal	Usually positive	None

Laboratory Examinations:

1. The cell count varies from normal to 2000 or more per cubic millimeter. They are predominantly lymphocytes.
2. The fluid is clear to turbid depending on the number of cells.
3. The protein level ranges from normal to 125 mg. per 100 cc.
4. The glucose level is normal.
5. The chlorides are normal or slightly reduced.
6. The colloidal-gold reaction shows a normal or Zone-II curve.

Guillain-Barre Syndrome:

This is also called "infectious polyneuritis" or "infectious neuronitis" and is apparently caused by a virus. The onset is suggestive of infection with facial diplegia and sensory disturbances.

Laboratory Examinations:

1. The protein level is increased and ranges from 100 to 800 mg. per 100 cc. in the cerebrospinal fluid.
2. The cell count is normal or low and ranges from 0 to 12. This dissociation between the cell count and protein level is characteristic of the disease.

TUMORS

Brain Tumors:

A comparative study of lumbar cerebrospinal fluid with ventricular fluid is of value in helping to localize the tumor.

Laboratory Examinations:

1. The lumbar fluid may be clear to xanthochromic.
2. The cell count varies from normal to 1000 or more per cubic millimeter. Lymphocytes predominate in the lower counts, and polymorphonuclear leukocytes are likely to predominate in the higher counts, especially in the presence of necrosis or hemorrhage.
3. The protein level varies from normal to 500 mg. per 100 cc., with an occasional case showing a higher value. According to Merritt and Smith,[2] when the lumbar fluid has a high-protein content, comparison with ventricular fluid aids in localizing the tumor as follows:
   - *a.* A normal-protein content of both ventricular fluids indicates that the tumor is in the posterior fossa, and the increased lumbar protein is due in part to block and stagnation of the

fluid in the lumbar sac and probably also to leakage from congested vessels in the neighborhood of the tumor, or from the tumor itself.

b. If both ventricular fluids have a high-protein level, it indicates that the tumor is in the third ventricle or the corpus callosum. Third-ventricle tumors cause increased protein in both lateral ventricles, perhaps by interfering with the circulation of the fluid through the foramina of Monro, or the increased protein may come from the tumor itself.

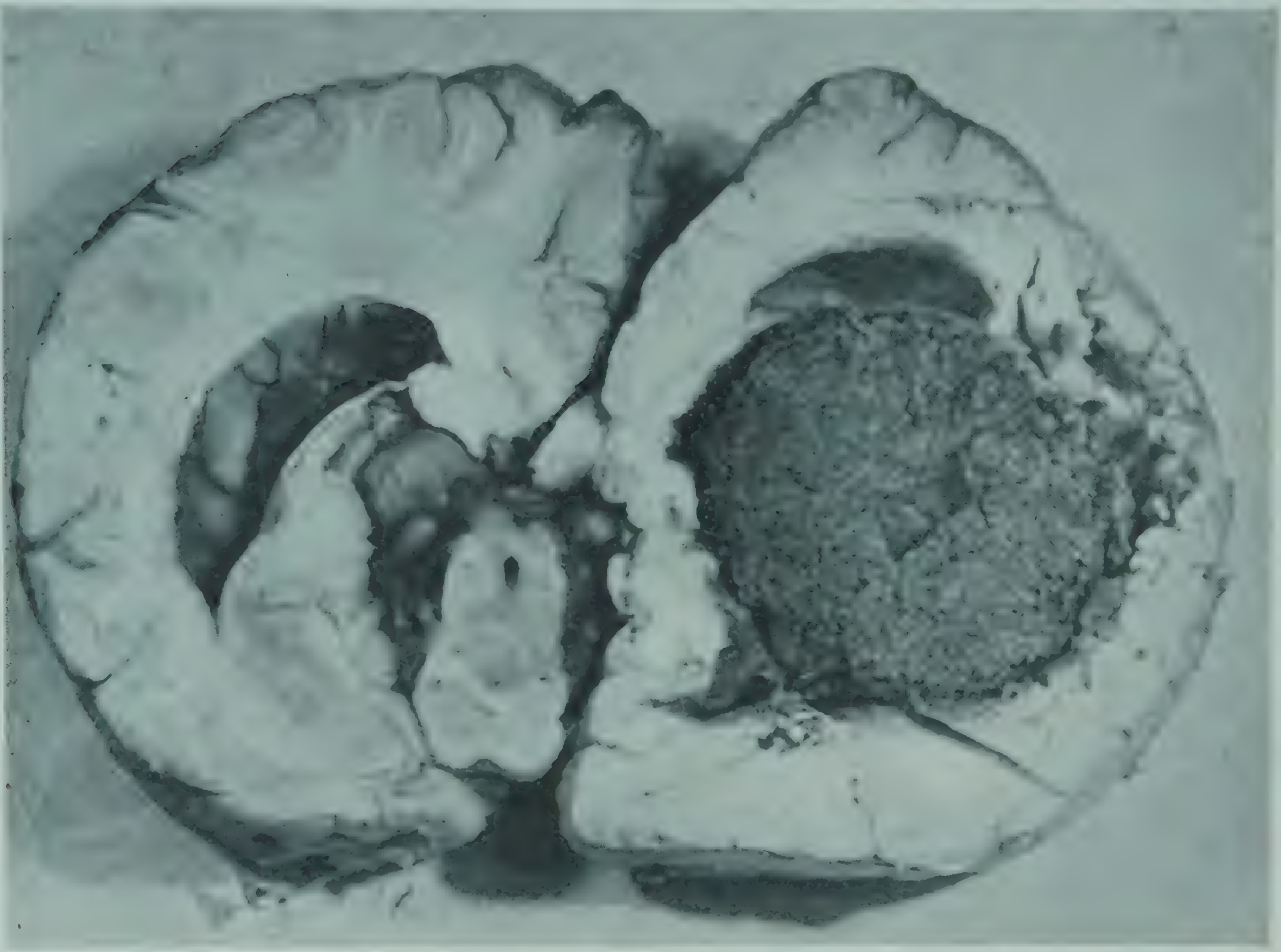

Fig. 1: Papilloma of choroid plexus. Cerebrospinal fluid was xanthochromic and contained red cells and increased total protein.

c. If the fluid from one lateral ventricle has a high-protein level while the other has a normal level, the tumor is on the side with the high protein and invades the wall of that ventricle or lies within it.

4. The glucose level is normal or slightly increased.
5. The chloride level is normal or slightly reduced.

Spinal-Cord Tumors:

Tumors of the spinal cord produce partial or complete block by pressure on the subarachnoid space. The diagnosis of block is diagnosed by noting a marked drop in pressure on removing lumbar fluid, by comparing the pressure with that of the cistern fluid, or by injecting lipiodol.

Laboratory Examinations:

1. The fluid is usually xanthochromic.
2. The protein level ranges from high normal to 100 mg. or more per 100 cc.
3. The cell count is normal in most cases, but it may be slightly increased. Lymphocytes predominate in the increased count.
4. The glucose level is normal or slightly increased.
5. The chloride level is normal or slightly reduced.
6. Examination of the sediment for tumor cells should be included.

TRAUMA

Injury of the head by a blow causes changes in the cerebrospinal fluid, which depend upon the extent of damage to the brain. Following such an injury, there may develop an epidural or subdural hemorrhage.

Laboratory Examinations:

1. The cerebrospinal fluid is usually frankly bloody if the brain is lacerated. The red-cell count is in proportion to the amount of hemorrhage. Red cells from hemorrhage are likely to be crenated.
2. The protein level is increased in proportion to the amount of hemorrhage and may reach 2000 mg. per 100 cc. The protein comes directly from the blood plasma and mixes with the cerebrospinal fluid.
3. In subdural hematoma, the fluid is xanthochromic. The total protein is markedly increased in the presence of blood but may be only slightly increased if the fluid is only xanthochromic.

SUBARACHNOID HEMORRHAGE

Spontaneous subarachnoid hemorrhage occurs as a result of the rupture of small aneurysms or of small veins.

Laboratory Examinations:

1. The fluid is bloody, and the red-cell count varies with the amount of hemorrhage. It ranges from 1000 to 3,000,000 or more per cubic millimeter. Some of the cells are usually crenated. Leukocytes are found in proportion to the red cells.

2. The protein level is increased and ranges from 100 to 1000 mg. or more per 100 cc.
3. The glucose level is normal, slightly increased, or slightly reduced.
4. The chloride level is normal or slightly reduced.
5. The supernatant fluid at first has only a slight yellow tinge but later becomes markedly xanthochromic.
6. If the hemorrhage stops, there is a progressive decrease in the red-cell count, white-cell count, and protein level. The color also gradually returns to normal.

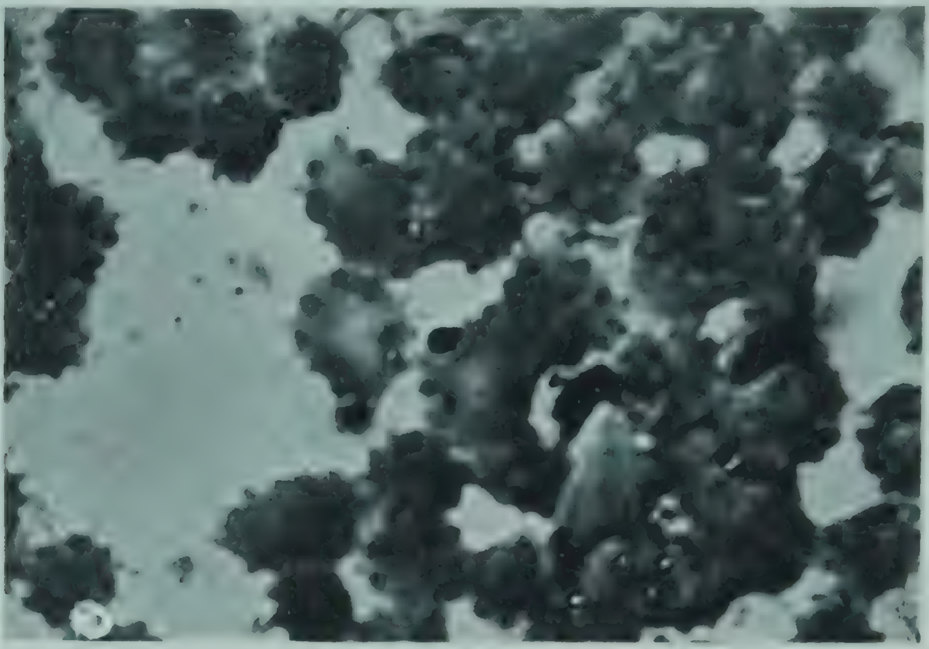

Fig. 2: Crenated red cells in cerebrospinal fluid.

RUPTURED DISK

Sciatic pain is sometimes caused by pressure on a nerve root by a ruptured intervertebral disk. The diagnosis of this condition is frequently difficult.

Laboratory Examinations:

The protein level is usually increased and ranges from 45 to 90 mg. per 100 cc. The protein level is helpful in those cases in which there is an increase, but unfortunately it is not always found.

MULTIPLE SCLEROSIS

The cerebrospinal fluid is normal in some cases of multiple sclerosis. The changes in the findings also do not parallel the activity of the disease, and no findings are considered pathognomonic.

Laboratory Examinations:

1. The cell count is slightly increased in some cases. When the count is increased, the cells are lymphocytes.
2. The protein level is increased to 100 mg. or slightly more in about one fourth of the cases.
3. The colloidal-gold reaction may show a Zone-I or -II curve, or there may be very little change in color.

CEREBRAL HEMORRHAGE AND THROMBOSIS

The differential diagnosis between cerebral hemorrhage and thrombosis is sometimes difficult, and in such cases examination of the cerebrospinal fluid is often helpful. The fluid pressure is usually higher in cerebral hemorrhage than in thrombosis, but this is difficult to evaluate in the individual case. A tabular comparison of the cerebrospinal-fluid findings in these two conditions follows:

Laboratory Examinations:

	Cerebral Hemorrhage	*Cerebral Thrombosis*
Appearance	Usually bloody; xanthrochromic after centrifuging	Clear
Red-cell count	1000 to 500,000 depending on amount of hemorrhage	0 to 500
White-cell count	Proportionate to red cells; may be increased later because of tissue necrosis	Normal or slightly increased due to area of necrosis impinging on ventricle
Protein	Level is high because of serum; later in xanthochromic fluid, it is only slightly increased	Normal or slightly increased
Glucose	Normal or slightly increased	Normal or slightly increased
Chlorides	Normal or slightly reduced	Normal or slightly reduced

INTRATHECAL INJECTIONS

The intrathecal injection of foreign substances causes an aseptic inflammatory reaction, which is reflected in the cerebrospinal fluid. The most significant changes are increased white-cell counts, the presence of red cells, and increased protein levels. These findings for the usual agents are summarized below.

Laboratory Examinations:

	Spinal Anesthesia	*Serum Injection*	*Air Injection*	*Lipoidal Injection*
Appearance	Clear	Turbid	Turbid	Turbid
Red-cell count	Negative	200 to a few thousands	200 to a few thousands	Negative
White-cell count	Increased to 100 or more for a few days	Increased to 100 or more	Increased to 100 or more	Increased to more than 500
Types of cells	Lymphocytes	Polymorphonuclears predominate	Polymorphonuclears predominate	Polymorphonuclears predominate
Protein	Normal or slight increase	Increased	Increased	Increased

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CHAPTER 27

Bacteriological Diseases and Diseases of Doubtful Cause

It is the object in this chapter to cover those infectious diseases that have not been completely discussed elsewhere and that lend themselves to laboratory diagnosis. In addition to the serological and bacteriological characteristics, which have been summarized in the appropriate chapters, this discussion gives all pertinent laboratory tests for the diagnosis of each disease.

SYSTEMIC INFECTIONS

UNDULANT FEVER (BRUCELLOSIS)

Undulant fever occurs as an acute or chronic infectious disease and is caused by one of the Brucella group of organisms. These organisms are divided into the following: (1) the bovine strain, Brucella abortus; (2) the caprine, or goat strain, Brucella melitensis; and (3) the porcine strain, Brucella suis.

Brucella abortus causes infectious abortion in cattle and mild undulant fever in man, who is usually infected through raw milk or milk products. This appears to be the most common cause of undulant fever in man in the United States.

Brucella melitensis occurs in goats and is transmitted to man through either the ingestion of raw goats' milk or handling infected goats. This strain, as well as Brucella abortus, has been found in the milk of infected cows. It is the most pathogenic strain for man.

Brucella suis rarely causes undulant fever in man. It was isolated by Traum[1] from aborted hog fetuses in this country and Europe. The hog is the natural host for Brucella suis, but it has been isolated also from the cow, dog, and fowl.

Undulant fever is protean in its clinical manifestations. Frequently, the patient has a remittent type of fever, sweats, chills, headache, joint and muscle pains, various nervous disorders, constipation, weakness, and secondary anemia.

Laboratory Examinations:

1. A positive blood culture establishes the diagnosis, but unfortunately such evidence is rarely found. Cultural methods, using carbon-dioxide tension as well as guinea-pig inoculation, should be tried.
2. The agglutination test is positive in the second or third week in the acute form of the disease. On repeated tests, an increasing titer is of particular diagnostic importance.

   An agglutination test may be positive as a result of a previous subclinical infection. Consequently, positive agglutination tests should be carefully evaluated in relation to the clinical picture.

   A negative agglutination test does not rule out undulant fever because some persons fail to develop a titer even with a positive blood culture.
3. Opsonocytophagic Index: The following interpretations are used at present: (1) opsonic index negative or slightly positive, that is 0 to 20, with negative skin test and negative agglutination reaction is indicative of susceptibility; (2) opsonic index negative, slight or moderate, that is 0 to 40, with positive skin test and with or without positive agglutination is indicative of infection, past or present; (3) opsonic index strongly positive, that is above 40, with positive skin test and with or without positive agglutination is indicative of immunity.
4. The intracutaneous skin test with Brucella antigen is positive. This test must also be carefully evaluated because it may be positive as a result of a previous infection. A negative skin test excludes Brucella infection past or present.

5. Leukopenia is usually present in those patients who have acute undulant fever. In the chronic form of the disease, the leukocyte count varies from a leukopenia to a moderate leukocytosis.
6. The differential count shows an increase in lymphocytes, both relative and absolute. The smear is also characterized by the presence of an appreciable number of immature lymphocytes.
7. There is a slight anemia, usually of the hyperchromic macrocytic type.
8. The sedimentation rate is only slightly increased.

TULAREMIA

Tularemia is primarily a disease of rabbits and hares, which is caused by Pasteurella tularensis. It is transmitted to man by flies and ticks, or directly by handling and dressing infected animals with bare hands. The infection is present in many wild animals, but rabbits and hares are of greatest importance, since they account for most of the infections in man. The disease is rarely contracted by ingestion of rare rabbit meat or through the respiratory tract in laboratory technicians who work with the organisms.

The incubation period varies from a few hours to three weeks, but averages two to five days. A skin lesion, or primary sore, develops at the site of infection, usually on the hands or exposed skin. Following this, there is a bacteremia for seven to ten days.

The following clinical types are found: (1) ulceroglandular type, (2) oculoglandular type, (3) glandular type, and (4) typhoid type. The ulceroglandular type is most common, while the others are rare. The usual onset is characterized by prostration, chills, fever, sweats, headache, general malaise, and sometimes nausea and vomiting.

The mortality rate is 5 to 6 per cent. Death is due to complications such as pneumonia, septicemia, or meningitis caused by the Pasteurella tularensis.

Laboratory Examinations:

1. Culture of the primary lesion with the isolation of Pasteurella tularensis establishes the diagnosis. However, it is difficult to culture the organism, and the presence of secondary invaders frequently confuses the picture.

2. Blood cultures should be done on more than one occasion for the isolation of the bacterium. Unfortunately, it grows slowly; thus, the diagnosis is frequently delayed four or five days.
3. Agglutination tests are positive after the first week and are perhaps most helpful in establishing a diagnosis. A rising titer is particularly significant. Normal persons may have an agglutination titer of 1:10 for Pasteurella tularensis. In a person with a history of exposure and with a clinical picture of the disease, a titer of 1:80 is significant. If this titer rises, it seems reasonable to make the diagnosis.

   Unfortunately, cross agglutination takes place between the antigens of Pasteurellae and Brucellae. It seems advisable, therefore, to test the agglutination of both organisms when either disease is suspected. It must be remembered that the intradermal skin test with Pasteurella-tularensis antigen causes a rise in the titer, possibly 1:40; therefore, the agglutination test should be done before the skin test.
4. The intradermal bacterial test introduced by Foshay[2,3] is positive in three or four days. This must be properly evaluated because previous infection with Pasteurella tularensis can cause a positive skin test. According to Friedewald and Hunt,[4] Foshay's intradermal test using antitularemic serum is not considered reliable because of nonspecific reactions.
5. The opsonocytophagic index is positive with Pasteurella tularensis. It is of particular value when there is cross agglutination with Brucella organisms.[4]
6. The leukocyte count is usually increased to 12,000 per cubic millimeter. The extremes are 3500 to 34,000 per cubic millimeter.

PLAGUE

Plague is an acute infectious disease caused by Pasteurella pestis. The organism in bubonic plague is spread from rat to rat, from rat to man, and from man to man by fleas. The flea that is most commonly incriminated is the Xenopsylla cheopis. The flea presumably infects human beings by depositing infected feces on the skin or by regurgitating infected blood in the course of biting.

Transmission by fleas does not account for the spread of pneumonic plague. Apparently, this is transmitted from man to man by coughing and spraying the organisms into the air.

The types of plague are as follows: (1) bubonic plague, which accounts for most of the cases; (2) ambulatory plague; (3) pneumonic plague; and (4) septicemic plague.

Laboratory Examinations:

1. Bacteriological examination of material from a primary lesion on the leg or of aspirated material from a bubo is the first diagnostic procedure. The organism (Pasteurella pestis) may be found by smear, culture, or guinea-pig inoculation. Sometimes other organisms, such as the streptococcus, are mixed with Pasteurella pestis.
2. In pneumonic plague the sputum is examined and cultured for Pasteurella pestis.
3. A blood culture should be carried out, although a septicemia is not always present.
4. The agglutination test is of little value because the titer does not rise above 1:40 and is sometimes negative in the presence of the disease.
5. The leukocyte count is markedly increased, and the proportion of polymorphonuclear leukocytes is increased.

GLANDERS

Glanders is an infectious disease of the respiratory organs or skin caused by Malleomyces mallei. It occurs primarily in animals of the equine group, such as horses or mules, and is transmitted to human beings by contamination of a scratch or by infection of the respiratory tract.

It occurs in both acute and chronic forms. In the acute form, it may cause a pustular eruption of the skin or a mucopurulent nasal discharge associated with marked prostration. It has a high mortality rate with death occupying under ten days. The chronic form is characterized by multiple subcutaneous abscesses, lymphadenitis, and coryza. Abscesses may occur in the bones, intestinal tract, and other viscera. If the patient survives, there is a tendency to quiescent periods with recurrence.

Laboratory Examinations:

1. Cultural examination of material from a pustule or nasal discharge may show Malleomyces mallei.
2. Blood culture should be carried out because it is sometimes positive in the acute form of the disease.
3. Inoculation of a male guinea pig with material or tissue from an ulcer causes acute orchitis (redness and swelling) in two or three days. The animal dies in about two weeks, and the autopsy reveals grayish nodules of the abdominal viscera, particularly

the spleen. For confirmation, the lesions should be examined by smear and culture.

4. The use of the complement-fixation test may be of help in diagnosing the disease in humans.
5. The skin test with Malleomyces-mallei antigen is of value in diagnosing latent glanders in horses and mules, but its value in man is not proved because of lack of experience.

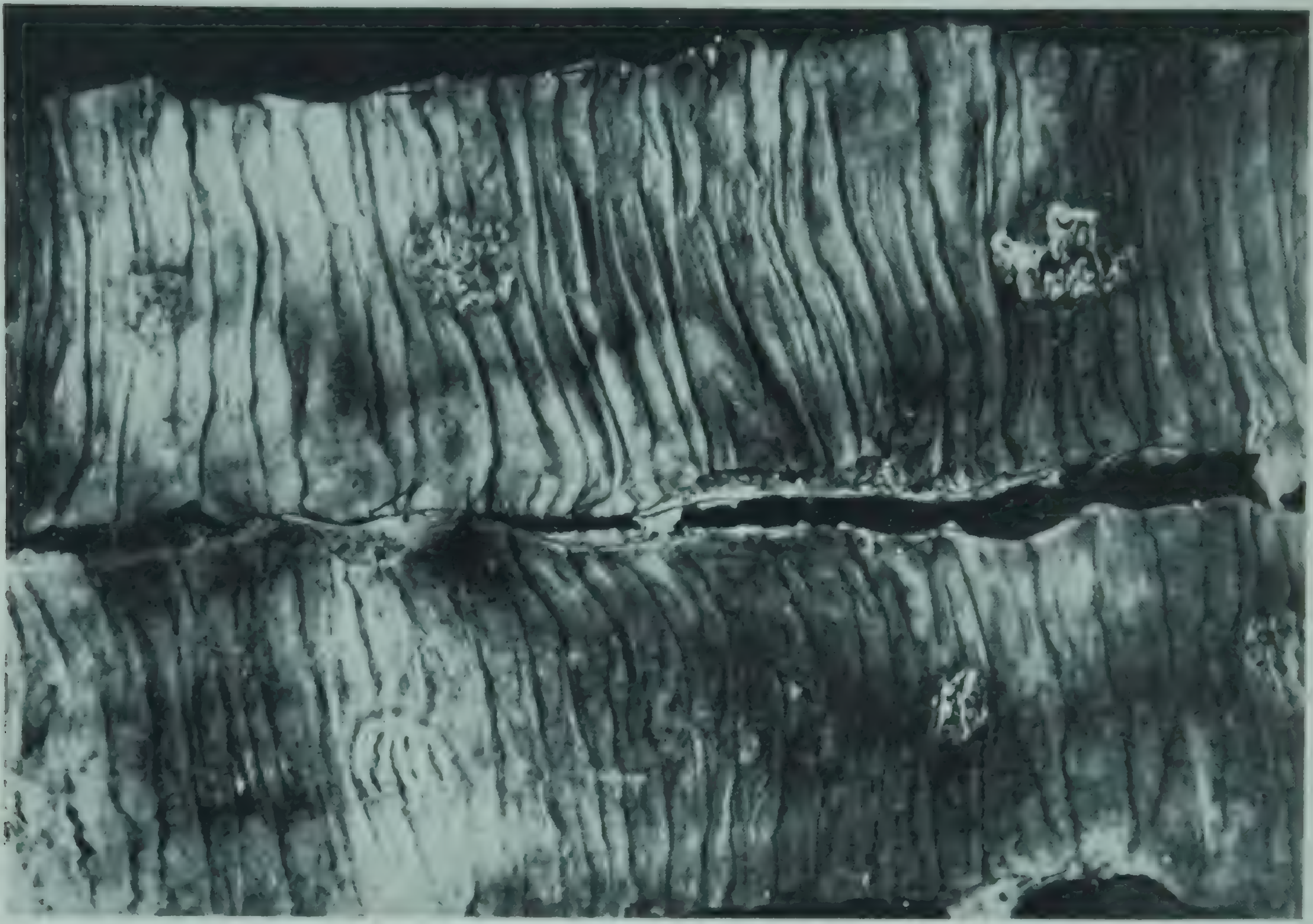

Fig. 1: Anthrax of intestine.

6. The agglutination test is not usually of value in making a diagnosis because of the delay in the development of a diagnostic titer in the acute form of the disease. In the chronic form, the titer is not increased or is only slightly increased above 1:100, which may be present in normal sera.
7. The leukocyte count is increased.

ANTHRAX

Anthrax is caused by a spore-forming organism, Bacillus anthracis. It occurs primarily in animals, such as cattle, sheep, and goats, and occasionally horses and hogs, and can be transmitted to man. In the United States, man is infected by handling skins and wool imported from abroad because of the greater prevalence of the disease in animals of foreign countries. The spores can survive on the skin and hair; and after entering the skin, and respiratory or intestinal tract of man, they start to grow. The mortality rate is extremely high (90 per cent) in pulmonary and intestinal anthrax. In cutaneous anthrax without septicemia, it may not exceed 20 per cent.

Laboratory Examinations:

1. Bacteriological examinations including smears and cultures should be carried out on material from cutaneous lesions, malignant pustules, and sputum of suspected patients who are skin and wool handlers. The smear stained by the Gram method is particularly helpful in making an early diagnosis of this disease.
2. Blood culture is also desirable in diagnosing the septicemic form.
3. The leukocyte count may be normal, slightly decreased, or slightly increased. When increased, the leukocyte count ranges from 10,500 to 13,500 per cubic millimeter.

LEPROSY

Leprosy is a chronic disease presumably caused by Mycobacterium leprae. The mode of transmission is not clearly understood. It is surprising that so few people working with lepers have contracted the disease. The organism may be carried by mosquitoes, flies, bedbugs, or other insects. The sites of entry into the human body are thought to be the skin, mucous membranes of the nose, and the gastrointestinal tract.

Clinical manifestations are disfiguring nodular lesions of the skin, particularly of the face and extremities. The lesions involve the skin, sensory nerves, or both. The incubation period is prolonged and has been estimated to be as long as six years or more.

Laboratory Examinations:

1. Examination of smears from the nasal mucosa stained by the Ziehl-Neelsen method for acid-fast bacilli is the most practical method of making a diagnosis. The organisms are present frequently in the nasal mucosa before the skin lesions appear.
2. Biopsy of a skin lesion yields acid-fast bacilli on smear or stained in situ in the tissue.

3. When it is necessary to differentiate these organisms from Mycobacteria tuberculosis, a guinea pig is inoculated. If the organisms are Mycobacteria tuberculosis, the guinea pig will die of tuberculosis, but the pig is not infected at all if they are leprosy bacilli.

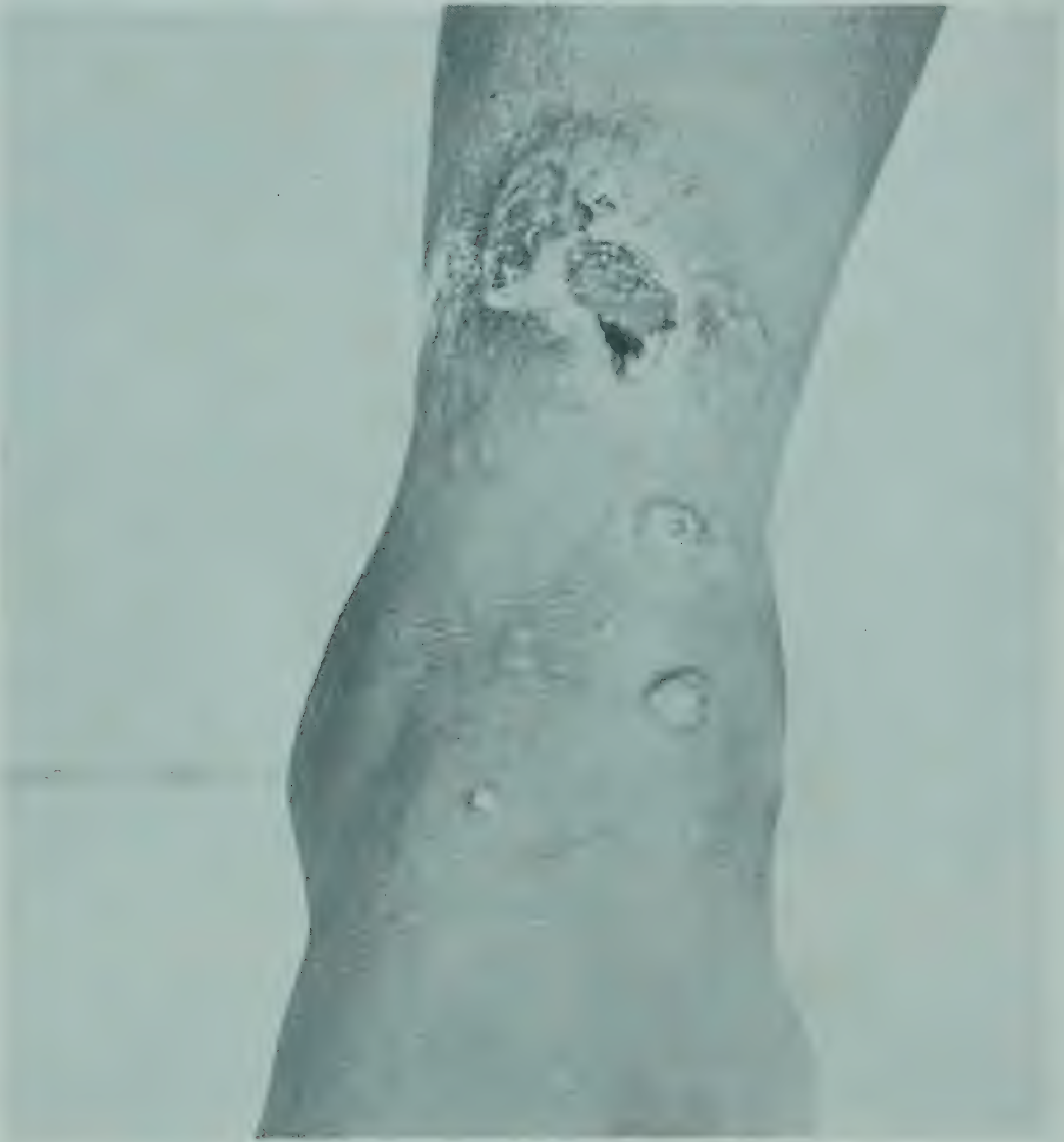

Fig. 2: Yaws of leg of eight-year-old boy.

4. A large number of leprosy cases have false or nonspecific, positive, flocculation and complement-fixation tests for syphilis.

SYPHILIS

Syphilis is discussed in its various forms in Chapters 3, 13, 24, and 26 (pp. 114, 269, 463, and 498).

YAWS

Yaws, a treponematosis, is apparently a nonvenereal disease caused by Treponema pertenue, which is morphologically indistinguishable from Treponema pallidum. Some have thought that it is a tropical form of syphilis, while others believe that it is a separate disease. Its occurrence in children in other sites than the genitals favors the latter. However, the relationship of the two diseases has not yet been clarified.

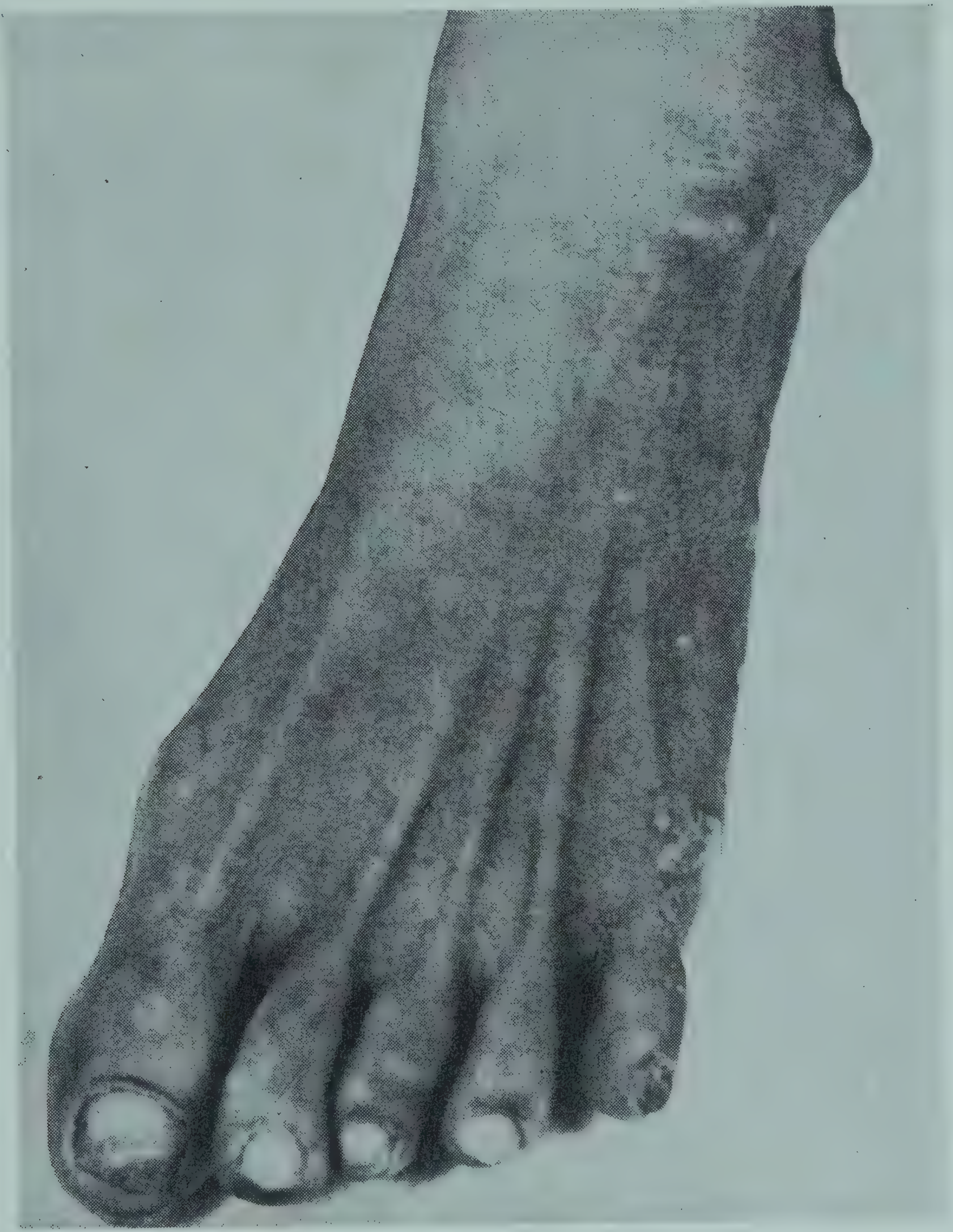

Fig. 3: Juxta-articular node of yaws.

The spirochetes are presumably transmitted from person to person by flies or gnats; thus transmission is favored by unsanitary conditions. It differs from syphilis in that the central-nervous-system and cardiovascular lesions do not occur. It does cause disfiguring gummalike lesions, particularly of the nose. Firm, nonnecrotic, subcutaneous nodules occur near the joints (juxta-articular nodes) in some cases.

Laboratory Examinations:

1. Darkfield examination of the skin lesion shows large numbers of spirochetes morphologically similar to Treponema pallidum.
2. Flocculation tests and complement-fixation tests are positive as in syphilis since the reagin is apparently the same. It is important to remember that leprosy, malaria, and jaundice may cause false-positive serological reactions.

BEJEL

Bejel is considered to be a nonvenereal form of syphilis that occurs among the Bedouin Arabs of Syria. Although it is similar to yaws, Hudson[5] hesitates to call it the same disease. The reservoir of the disease is in the children, who transmit it to each other and to adults.

The disease is characterized by grayish oral patches and papules about the genitalia and folds of the skin and sometimes on the trunk. The lesions in the mouth may extend to the bones of the nose, producing softening and collapse of the nasal bridge. In the course of a year, these lesions disappear, leaving a healthy child.

Before the advent of antibiotics, an injection or two of an arsenical or bismuth preparation abolished the lesions. However, the children are not usually brought in for treatment, since the parents know it is self-limited.

Laboratory Examinations:

1. Darkfield examination of the lesions reveals spirochetes indistinguishable from Treponena pallidum.
2. The Kahn test is positive in about 60 per cent of the cases.

PINTA

Pinta, sometimes called "vitiliginous yaws," is a chronic cutaneous disease presumably caused by a spriochete (Treponema carateum). It is not known at present whether this is a stage of yaws or a separate entity. It is characerized by irregular areas of white skin, particularly of the hands and feet.

Laboratory Examinations:

1. The flocculation and complement-fixation tests for syphilis are positive in the majority of cases.

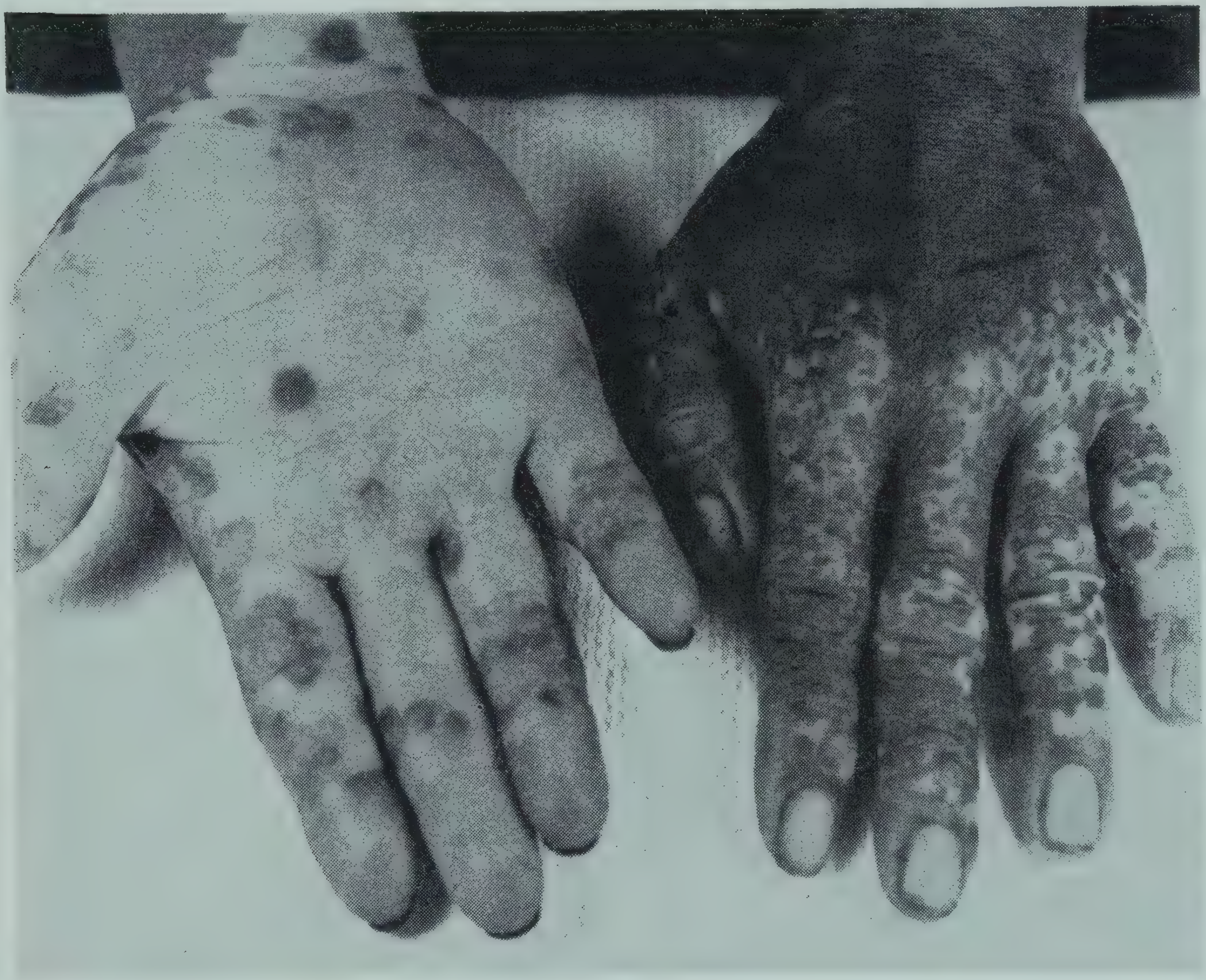

Fig. 4: Pinta of hands and fingers.

2. Darkfield examination of scrapings from the lesions is not satisfactory.

RELAPSING FEVER

Relapsing fever is an acute infectious disease caused by a group of spirochetes that have different immunological characteristics but are morphologically similar. They are Borrelia recurrentis, Borrelia duttoni, Borrelia carteri, and Borrelia novyi. The spirochetes are carried from person to person by lice and ticks.

The disease is characterized by fever, chills, general malaise, muscle aches, prostration, jaundice, and enlarged spleen. The symptoms subside after three to ten days, but a relapse may take place after one to three weeks.

Laboratory Examinations:

1. The blood is examined by stained smear or darkfield for spirochetes. Between attacks, they cannot be found, but with the next attack they can usually be demonstrated.
2. When spirochetes cannot be found by direct examination, intraperitoneal inoculation of a guinea pig may prove the presence of the organisms.

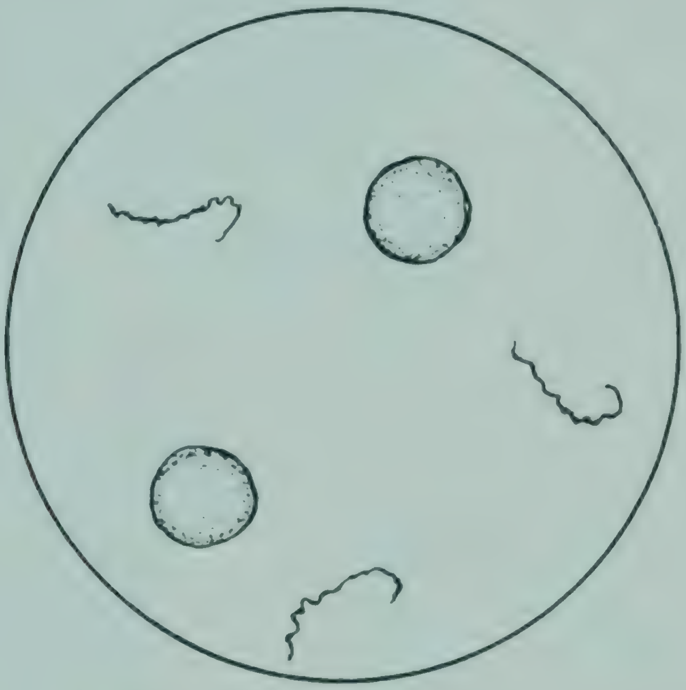

Fig. 5: Leptospira icterohemorrhagiae.

LEPTOSPIROSIS

The most common form of leptospirosis is spirochetal jaundice (Weil's disease), which is caused by Leptospira icterohaemorrhagiae and occasionally by Leptospira canicola. The infection occurs in wild rats, field mice, dogs, and other animals. Man is infected by contact with the excreta of these animals, particularly the urine of wild rats.

The incubation period is six to twelve days. The disease is characterized by jaundice, chills, fever, muscular pains, headache, conjunctival congestion, intestinal symptoms, and purpura. About one third of the patients die; those who recover develop an acquired immunity.

Other leptospiral diseases are found in certain parts of the world and have very little if any jaundice. They are as follows:

Name of Disease	*Type of Leptospira*	*Location*
Swamp fever	Leptospira grippotyphosa	Parts of Russia and southern Bavaria
Seven-day fever	Leptospira hebdomidis	Japan
Hasami fever	Leptospira autumnalis	Japan
Panama fever		Australia

Laboratory Examinations:

1. Examination of the blood by darkfield may reveal the Leptospirae. Care should be used in interpreting Leptospiralike bodies protruding from the red cells. These are usually fibrin artefacts.
2. Blood culture should be carried out for identification of the Leptospirae.
3. Intraperitoneal inoculation of guinea pigs with blood is important if the darkfield is negative.
4. Intraperitoneal inoculation of guinea pigs with urine after the first week is advisable, since the leptospirae are excreted in the urine after this time. The kidneys and liver are examined for Leptospirae by silver stain.
5. Normal humans agglutinate both Leptospira icterohemorrhagiae and Leptospira canicola in dilutions up to 1:40. In persons infected with one of the Leptospirae, the titer increases rapidly after the fifth day up to 1:100,000 and lasts for about two months, after which it decreases.
6. The mouse-protection test and agglutination-lysis test are supplementary diagnostic procedures that may be used.
7. The icterus index and quantitative van den Bergh are increased. The qualitative van den Bergh shows a direct reaction.
8. Examination of the urine reveals albumin, red cells, and casts, which are the result of the presence of the Leptospirae in the kidneys.

LISTERELLOSIS

Listerellosis is an acute infectious disease of rabbits and guinea pigs caused by Listerella monocytogenes, a gram-positive motile bacillus.[6] It produces a suppurative meningitis in man,[7,8] which has a mortality rate of approximately 70 per cent.

Laboratory Examinations:

1. A Gram stain of a smear of the cerebrospinal fluid shows intracellular and extracellular gram-positive bacilli. Those that are intracellular are found in monocytes.
2. The cell count is increased to several hundred, and monocytes predominate.
3. Further examination of the cerebrospinal fluid reveals an increase in the total protein and a decrease in the glucose level.
4. Examination of the blood reveals a leukocytosis with an increase in monocytes.
5. According to Pons and Julianelle,[9] the blood culture may be positive in severe cases.
6. This organism is not found in the blood in infectious mononucleosis, although it has been suggested as the causative agent.

INFECTIONS OF INTESTINES

Typhoid Fever

Typhoid fever is an acute infectious disease of man caused by the Eberthella typhosa, which is found in the bloodstream as well as in the intestines (Peyer's patches), urine, and bile. The organism gains entrance to the human body by the ingestion of water, food, or milk that has been contaminated.

The disease is characterized by fever, headache, prostration, diarrhea or constipation, splenomegaly, and leukopenia. The hyperplastic Peyer's patches undergo ulceration about the third week. At this time, the danger of hemorrhage or perforation is present. The Eberthellae typhosae are now found in the feces, since they are present in the necrotic material from the ulcerating patches or lymph nodes.

Various complications are seen, such as liver necrosis, periostitis, arthritis, meningitis, and infection of the gallbladder in carriers.

Laboratory Examinations:

1. A blood culture usually contains the organism in the first week of the disease. By the third week, only about one third of the cases have positive blood cultures.
2. Examination of the feces for Eberthella typhosa is positive in only 10 to 15 per cent of cases in the first week. After the Peyer's patches break down in the third week, the percentage rises rapidly so that during convalescence as many as 90 per cent of the stools contain Eberthella typhosa.

Repeated cultures of the stools are necessary to detect those who are carriers. It has been found that 2 to 4 per cent become chronic carriers with the focus in the gallbladder.

3. Agglutination tests are particularly helpful after the first week of the disease. However, they may not be significant at first and require repetition. False-positive agglutinations may occur as a result of a previous attack of the disease or following immunization.

   Both O (somatic) and H (flagellar) antigens should be used in testing the sera. An increase in titer above that expected in normal nonimmunized persons is significant. These titers for O and H antigens may be tabulated as follows:

Antigen	*Normal*	*Typhoid Fever in Nonimmunized Persons*	*Typhoid Fever in Previously immunized Persons*
O (somatic)....	Up to 1:100	1:160 or higher	Progressive increase
H (flagellar)...	Up to 1:20	1:80 or higher	1:2180 or higher

   Typhoid carriers usually have a titer above 1:100 for the O antigen and 1:20 for the H antigen.

4. The complement-fixation test is specific and is helpful in the diagnosis of typhoid fever, even in those persons previously immunized.
5. Culture of the urine for Eberthellae typhosae is not usually necessary for diagnosis. After the second week of the disease, less than 30 per cent of cases show organisms in the urine. Before discharging the patient, it is necessary to make sure that no Eberthellae typhosae are being excreted in the urine, since an occasional chronic carrier may eliminate organisms by this route.
6. A leukopenia is characteristic of typhoid fever, although a normal leukocyte count occurs. The differential count has an increase in lymphocytes and a decrease in neutrophils. A leukocytosis during the third week suggests perforation with peritonitis.
7. An anemia of the hypochromic normocytic type is common.
8. Ehrlich's diazo reaction is usually positive from the fifth to twenty-first day of the disease. In typhoid fever, the urine contains phenol or a phenol derivative, which reacts with Ehrlich's diazo reagent to produce a red color. Repeated negative tests between the fifth and twenty-first day serve to rule out typhoid fever in a problem case.

PARATYPHOID FEVER

Paratyphoid fever is an acute infectious disease caused by bacteria of the Salmonellae group, particularly Salmonella paratyphi (A) and Salmonella schottmülleri (B). It is similar to typhoid fever, but is usually milder and of shorter duration. The organisms are transmitted by food, milk, or water.

Laboratory Examinations:

1. Repeated blood cultures during the first week of the disease are helpful in making a diagnosis.
2. Cultural examination of the feces frequently reveals the causative organism after the second week. It may be one of the paratyphoid group or some other Salmonella organism.
3. Agglutination tests are carried out routinely, but they do not have the diagnostic value of similar tests in typhoid fever. The O and H antigens are used for both Salmonella paratyphi and Salmonella schottmülleri. An increase in titer above that expected in normal nonimmunized persons is significant. These titers for O and H antigens may be tabulated as follows:

Antigen	*Normal*	*Paratyphoid Fever (Salmonella paratyphi and Salmonella shottmülleri)*
O (somatic)	Up to 1:20	1:80 or higher
H (flagellar)	Up to 1:80	1:160 or higher

   There may be some increase in cross agglutination with Eberthella typhosa, but it does not approach the titer for paratyphoid organisms.

   In a person previously immunized against paratyphoid fever, the titer may not be diagnostic. In such a case, the diagnosis depends upon increasing agglutinin titer for the O antigen of the particular organism involved.

4. There is usually a leukopenia with an increase in the number of lymphocytes.

BACILLARY DYSENTERY

This is an acute infectious disease of the intestine characterized by frequent stools containing blood and mucus, abdominal pain, tenesmus, and prostration. It is caused by a variety of dysentery bacilli of the genus Shigella.

Laboratory Examinations:

1. Culture of the fresh stool is the only unequivocal way of determining the causative agent of dysentery. In fact, culture is the only method of making a diagnosis if direct microscopic examination fails to reveal parasitic causes of dysentery such as Endamoeba histolytica and Balantidium coli.

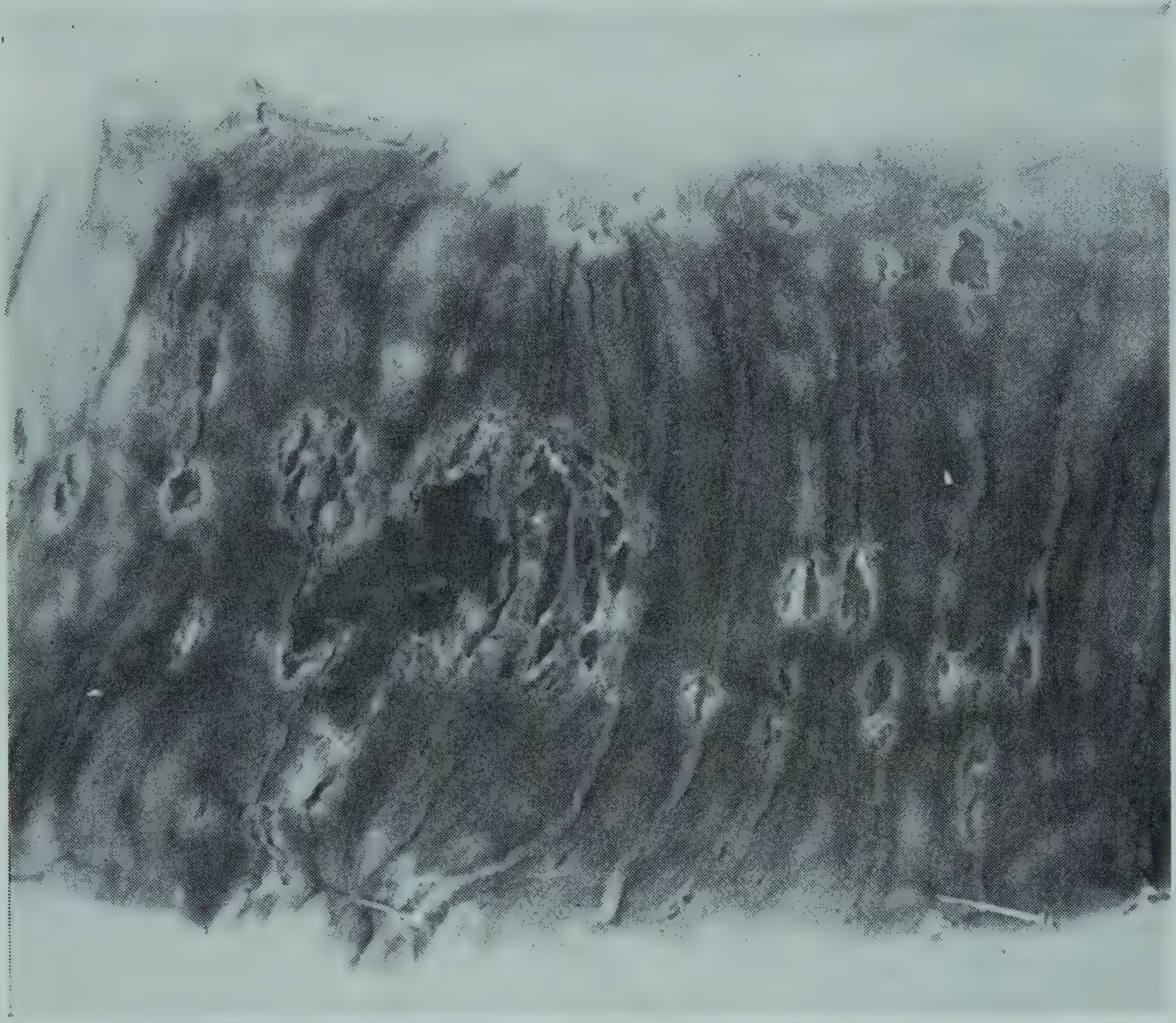

Fig. 6: Section of colon showing ulcers of bacillary dysentery.

2. Microscopic examination of the stools reveals many pus cells, epithelial cells, lymphocytes, and macrophages.
3. Agglutination tests are helpful as a supplementary diagnostic procedure. The normal and significant titers of the important dysentery bacilli may be summarized as follows:

Dysentery Bacillus	*Normal Titer*	*Titer in Active Dysentery*
Shigella dysenteriae	Up to 1:20	1:40 or higher
Shigella of Flexner	Up to 1:150	1:300 or higher
Shigella sonnei	Up to 1:50	1:100 or higher
Newcastle bacillus	Up to 1:128	1:256 or higher

4. Blood cultures may be positive in severe acute infections.

SALMONELLA INFECTIONS

Salmonella infections are caused by the ingestion of food containing bacilli of the Salmonella groups. The symptoms are usually those of acute gastroenteritis; but in the case of some organisms, such as the Newcastle bacillus, the symptoms suggest bacillary dysentery.

Laboratory Examinations:

Cultural examination of the fresh stool is most important for the isolation of the causative organism. The antigenic classification of members of the Salmonella group is very complex and commands the service of a bacteriologist experienced in this type of work.

FOOD POISONING

Food poisoning, or food intoxication, is caused by the ingestion of bacterial toxins in inadequately cooked foods. The two important organisms whose toxins cause symptoms of food intoxication are Clostridium botulinum and Staphylococcus aureus.

Laboratory Examinations:

1. Cultural examination of the feces is carried out only to rule out bacterial causes of the diarrhea.
2. Cultural examination of the suspected food may reveal the organism. The coagulase test should be done to prove the virulence of the staphylococcus.

CHOLERA

Cholera is an acute infectious disease, occurring in epidemics and caused by the Vibrio comma. The disease is characterized by a painless watery diarrhea, vomiting, muscle cramps, prostration, and ultimate collapse. Extreme dehydration develops because of the large amount of fluid lost in the stools.

The organism is transmitted by the ingestion of contaminated food or drink. The contamination may come from another patient or from a carrier.

Laboratory Examinations:

1. Bacteriological examination of the feces furnishes conclusive evidence of the disease when the cholera Vibriones are found.
2. The rice-water stools are characteristic. This whitish appearance is due to the presence of epithelial flakes in a watery medium.
3. Agglutination tests are of little value because of insufficient time for the production of agglutinins. Normal persons can agglutinate the Vibriones in a dilution of 1:10, while immunized persons and carriers have higher titers.
4. Evidence of dehydration is present in the form of hemoconcentration, increased total protein, acidosis, decreased blood chlorides, suppressed urinary excretion, and sometimes uremia.

ARTHRITIS

A complete history, physical examination, and laboratory investigation are essential in order to make a correct diagnosis in the arthritic patient. In some cases, the diagnosis can be confirmed by one or more laboratory tests; in others, trial with specific medication is necessary; and in a few cases, only repeated observations and the passage of time will give a final diagnosis.

Most cases of arthritis come under one or more of the following categories:

1. Infectious (caused by specific bacteria)
2. Probably infectious, but with etiology not proved
3. Degenerative joint disease (hypertrophic arthritis or osteoarthritis)
4. Traumatic joint disease
5. Gout

The following classification of arthritis is modified from that given by Jordan and associates.[10] It is printed with the permission of the Journal of the American Medical Association:

1. Arthritis due to infection with gonococcus, tubercle bacillus, pneumococcus, T. pallidum, typhoid and paratyphoid bacillus, meningococcus, staphylococcus, streptococcus, and fungus; also arthritis associated with dengue fever, dysentery, chronic ulcerative colitis, leprosy, malaria, brucellosis, scarlet fever, yaws, diphtheria, B. coli infections, B. influenza infections, and some forms of tenosynovitis
2. Arthritis due to rheumatic fever

3. Arthritis, rheumatoid
    a. Arthritis, rheumatoid, multiple (synonyms: proliferative arthritis, atrophic arthritis, chronic infectious arthritis, and Still's disease)
    b. Arthritis, rheumatoid, atypical (focal infectious arthritis)
    c. Arthritis, rheumatoid of spine (synonyms: rheumatoid spondylitis, atrophic spondylitis, Marie-Strumpell disease, spondylose rhizomalique, infectious spondylitis, spondylitis ankylopoietica, and von Bechterew's disease)
4. Arthritis due to trauma
5. Neurogenic arthropathy
6. Arthritis due to gout
7. New growths of joints
8. Degenerative joint disease (synonyms: osteoarthritis, hypertrophic arthritis, degenerative arthritis, and chronic senescent arthritis)
9. Mixed forms (especially rheumatoid arthritis and degenerative joint disease)
10. Hydrarthosis
11. Periarticular fibrositis

The following diseases are associated with arthritis, arthopathy, or arthralgia:

Acromegaly
Acute disseminated lupus erythematosis
Cyst of meniscus of knee
Dermatomyositis
Drug intoxication
Erythema multiforme exudativum
Erythema nodosum
Hemophilia
Hysteria
Ochronosis
Osteochondritis dissecans
Osteochondromatosis
Periarteritis nodosa
Psoriasis
Raynaud's disease
Reiter's disease
Scleroderma
Serum sickness
Purpura

NORMAL SYNOVIAL FLUID

For comparison with abnormal synovial fluids, the following values of normal synovial fluid are given:

Leukocyte count: 200 cells or less per cubic millimeter
Differential count:[11]
    Monocytes: 48 per cent
    Lymphocytes: 25 per cent

Polymorphonuclear leukocytes: 7 per cent
Clasmatocytes: 10 per cent
Unclassified phagocytes: 5 per cent
Synovial cells: 3 per cent
Unclassified cells: 2 per cent
Eosinophils or basophils: usually none

Erythrocytes: none
Culture: negative
Total protein: less than 2.5 per cent;[12] albumin-globulin ratio varies
Nonprotein nitrogen: 20 to 40 mg. per 100 cc.
Fibrinogen, cholesterol, or fatty acids: none
Glucose: less than in blood
Mucin: 0.14 to 0.85 per cent; mucin is responsible for osmotic pressure
*p*H: 7.4
Specific gravity: 1.008 to 1.015; average 1.010
Viscosity: 2.8 to 400; average 150

ARTHRITIS DUE TO INFECTION

Laboratory Examinations:

1. Bacteriological examination of material removed from the involved joint will reveal the causative organism, which is usually one of those mentioned above.

   If the Mycobacterium tuberculosis is suspected, guinea-pig inoculation and biopsy should be included.
2. The joint fluid is cloudy due to large numbers of polymorphonuclear leukocytes, except in tuberculous arthritis where there is an increase in lymphocytes.
3. Blood cultures are rarely positive in gonococcal arthritis but are more frequently positive in streptococcal, staphylococcal, pneumococcal, and meningococcus types.
4. The sedimentation rate is increased in all forms.
5. There is a leukocytosis with a Shilling shift to the left in the streptococcal, staphylococcal, pneumococcal, meningococcal, and gonococcal types of arthritis, and in those caused by Hemophilus influenzae and mycobacterium tuberculosis.
6. The antistreptolysin titer and streptococcal agglutinins are increased in streptococcal arthritis.
7. Specific agglutination titers are increased in brucellar and typhoidal arthritis.
8. A normocytic hypochromic anemia is usually present in all forms.
9. The gonococcal, complement-fixation test is positive in most cases of gonorrheal arthritis.

10. Specific flocculation and complement-fixation tests are positive in practically all cases with a syphilitic background.

    In arthitis associated with dengue fever, dysentery, chronic ulcerative colitis, leprosy, malaria, brucellosis, scarlet fever, yaws, diphtheria, and infections with Escherichia coli or Hemophilus influenzae, the underlying disease must be diagnosed first so that appropriate therapy can be instituted.

ARTHRITIS DUE TO RHEUMATIC FEVER

Laboratory Examinations:

1. Antistreptolysins, precipitins, and antifibrinolysins are positive Streptococcal agglutinins are found occasionally.
2. The sedimentation rate is increased.
3. The leukocyte count is increased, with a shift to the left of the Shilling index in the acute stage.
4. The Skin test with Streptococcus hemolyticus is usually positive.
5. The formol-gel reaction is positive in most cases in the presence of active carditis.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a nonspecific infectious form, the cause of which is not completely known. Theories of etiology include the following: focal infection, bacterial allergy, virus infection, vitamin deficiency, metabolic disorders (including food allergy), trauma, circulatory disturbances, and neurogenic and endocrine disorders.

Bacterial allergy with or without other factors, such as poor heredity, poor environment, endocrine imbalance, dietary and vitamin deficiency, or exposure to cold, may be the background for the development of this type of arthritis.

It is characterized by fever, symmetrical joint involvement with joint pains, swelling, stiffness, and progressive muscular atrophy. The joints involved are the proximal interphalangeal, metacarpophalangeal, toes, wrist, knee, elbow, ankle, shoulder, hip, temporomandibular, and sternoclavicular.

Laboratory Examinations:

1. The sedimentation rate is usually moderately to markedly increased.
2. Serological tests:
   a. Antistreptolysin titer is increased in early stage of the disease.

   b. The differential sheep-cell-agglutination test is positive in about half of the cases.
   c. Antifibrinolysin is present in the early stage.
3. The formol-gel reaction is usually positive in the active stage.
4. A leukocytosis of 12,000 to 20,000 per cubic millimeter is noted in the acute stage.
5. The polymorphonuclear neutrophils average 64 per cent. Band forms are increased to 16 per cent or more. Slight eosinophilia may occur.
6. Moderate to marked hypochromic anemia is frequently present.
7. In the synovial fluid, the cell count is increased, and polymorphonuclear leukocytes predominate.
8. Blood cultures are usually negative, but the results reported are variable.

GOUT

Gout is thought to be a hereditary constitutional disease that occurs in males in over 95 per cent of the cases. The cause is not known, but the high blood-uric-acid level is evidence of the inability of the patient to dispose of uric acid.

It is characterized by swelling and pain in the great toe in 70 per cent of cases in the initial attack and in 90 per cent later. Attacks are recurrent with complete remissions until joint changes take place. Tophi are present in about half the cases and are usually painless.

Laboratory Examinations:

1. Blood uric acid is elevated in about 75 per cent of patients with gout. (Uric acid is present in the blood in the form of sodium biurate.) If there is any question of nitrogen retention from impaired kidney function, a blood urea nitrogen should be done with the test for uric acid.
2. The contents of tophi are made up of sodium biurate, which gives a positive murexide (Weidel) test.
3. The sedimentation rate is usually elevated during attacks and is normal between attacks.
4. A moderate leukocytosis occurs during attacks.
5. The phenolsulfonphthalein, kidney-function test shows evidence of renal impairment in late stages.
6. Nitrogen retention is also present in the late stages.

OTHER TYPES

The remaining types of arthritis do not lend themselves to laboratory diagnosis except as far as the negative tests rule out the arthritides discussed above. However, in certain types, such as hemophilic arthritis, laboratory tests aid in the diagnosis of the underlying disease.

COLLAGEN DISEASES

A few diseases which have changes in collagen have been grouped as the collagen diseases. This does not mean that they have a common cause, but it does mean that they have specific changes in the connective tissue in various parts of the body. Connective tissue may react to injury by necrosis, fibrinoid degeneration, cellular proliferation and infiltration, and sclerosis. Although the pathological changes common to all diseases of this group are not necessarily evidence of a common cause, they do explain the clinical similarities between the diseases of this group.

The diseases which are usually included in this group are rheumatic fever, rheumatoid arthritis, polyarteritis (periarteritis nodosa), disseminated lupus erythematosus, scleroderma, and dermatomyositis.

Rheumatic Fever

See pp. 388 and 532.

Rheumatoid Arthritis

See p. 532.

Periarteritis Nodosa

See p. 394.

Disseminated Lupus Erythematosus

This is a disease with a long clinical course, characterized by a prolonged irregular fever, with a tendency to remissions, recurrent involvement of synovial and serous membranes (polyarthritis, pleuritis, pericarditis), depression of bone-marrow function, evidence of vascular alterations in the skin, retina, kidneys, and other viscera, and sometimes an erythematous rash.

Laboratory Examinations:

1. There is leukopenia varying from 3500 to 5000 cells per cubic millimeter.
2. There is moderate secondary anemia with a corresponding hemoglobin.
3. The platelets are reduced, sometimes producing purpuric manifestations.
4. The total protein may be increased during exacerbations with an increase in globulin.

5. The sedimentation rate is increased.
6. Lupus erythematosus cells ("L.E. cells") are found in stained bone-marrow smears. These cells are usually neutrophilis, containing masses of purplish-staining chromatin which fills the entire cell and pushes the nucleus to one side.

   These cells may be developed in neutrophils from normal persons by incubating the buffy coat or bone marrow with the plasma from a patient with active lupus erythematosus.
7. A serological test for syphilis sometimes gives a false-positive result.

SCLERODERMA

This is a chronic progressive disease characterized by a peculiar induration and rigidity of the skin.

Laboratory Examinations:

The laboratory findings are not diagnostic but the following changes are found:

1. The urine shows albumin.
2. There is moderate anemia.
3. There is slight increase in serum globulin.
4. The sedimentation rate is slightly increased.

DERMATOMYOSITIS

This is a chronic nonsuppurative inflammation of the skin and muscles characterized by insidious onset, progressive weakness, dermatitis, and tenderness of various muscles.

Laboratory Examinations:

The laboratory findings are not diagnostic, but the following changes are found:

1. The urine shows albuminuria and red cells in the sediment.
2. There is moderate anemia.
3. The white-cell count is not significant, but there is eosinophilia in about 25 per cent of cases.
4. The sedimentation rate is markedly increased.
5. The serum globulin is increased with a reversal of the albumin-globulin ratio.
6. A muscle biopsy is frequently performed to confirm the diagnosis, although the pathological findings are not always specific. At least, it should rule out trichinosis.

SARCOIDOSIS

Sarcoidosis, also known as "benign lymphogranuloma," is a chronic relapsing form of reticuloendotheliosis. The cause has never been definitely established. The first finding may be symptomless enlargement of superficial lymph nodes, a benign skin eruption, or abnormal shadows in an x-ray film of the lungs.

Laboratory Examinations:

1. A biopsy of a lymph node will confirm the diagnosis.
2. The total protein is increased to from 8 to 10 gm. per 100 cc. The increase is due chiefly to an increase in gamma globulin.
3. The white-cell count is usually normal, but the monocytes and eosinophils are frequently increased.
4. The sedimentation rate is increased.
5. The alkaline phosphatase is increased, sometimes to 17 Bodansky units.
6. The blood calcium is increased in some cases up to 16 mg. per 100 cc.

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CHAPTER 28

Vitamin Deficiency

Specific diseases have been described that are now known to be due to deficiencies of certain vitamins. Sometimes, more than one vitamin is deficient, and the clinical picture is confusing. Vitamin deficiencies may develop as a result of inadequate intake, or improper absorption and utilization of vitamins. The onset of vitamin deficiency is insidious and slow. The diagnostic signs and symptoms may not become manifest for months or years.

For those who are familiar with vitamin-deficiency diseases, the diagnosis is not usually difficult from the history and physical examination. Laboratory examinations sometimes confirm the clinical diagnosis, particularly when the level of the particular vitamin can be determined.

VITAMIN-A DEFICIENCY

Vitamin A is formed from carotenes by the liver and stored there. It is a fat-soluble vitamin that is a constituent of visual purple, maintains the normal status of the epithelium, and affects calcium metabolism. Deficiency of vitamin A (see p. 242) causes xerophthalmia, follicular keratosis, night blindness, bony metaplasia, and the formation of renal calculi.

Laboratory Examinations:

1. Vitamin-A deficiency is detected most efficiently by biophotometric examination of the eyes.
2. The urine or serum may be examined by comparing the blue color produced by vitamin A and antimony trichloride with a standard, copper-sulfate solution as described by Carr and Price.[1] According to the technic of Pett and LePage,[2] the normal range is from 35 to 40 international units.

VITAMIN-B DEFICIENCY

The vitamin-B complex is made up of a number of substances, but the functions of all of them are not yet known. These substances include thiamine chloride, nicotinic acid, riboflavin, pyridoxine, pantothenic acid, biotin, inositol, choline, and *para*-aminobenzoic acid. Deficiency of certain of these factors causes clinical pictures that are quite characteristic.

Beriberi:

This disease is due to a deficiency of thiamine chloride or vitamin B. Such a deficiency results in multiple neuritis, anasarca, cardiac failure, or a combination of these.

Laboratory Examinations:

1. The thiamine level in the blood and urine is decreased. The test is based upon the thiochrome reaction as described by Peters,[3] by which the fluorescence of partially oxidized thiamine is measured.
2. The albumin-globulin ratio is reversed.
3. A hyperchromic type of anemia is usually present.

Pellagra:

This disease is due primarily to a deficiency of nicotinic acid, but riboflavin (B_2) deficiency is probably a contributing factor. It is characterized by diarrhea, dermatitis, and dementia.

Laboratory Examinations:

1. Nicotinic acid is decreased in the blood and is absent in the urine. The colorimetric determiniation of nicotinic acid is described by Peason,[4] and Melnick and Field.[5] The normal range is from 0.1 to 0.3 mg. per 100 cc. of plasma.

Rosenblum and Jolliffe[6] give a simple method for estimating the urinary excretion of nicotinic acid. The normal excretion is 3.5 to 10.0 mg. in twenty-four hours.

2. There is excessive excretion of porphyrin or porphyrinlike substances in the urine according to Beckh and co-workers.[7]
3. When dehydration supervenes from diarrhea and vomiting, there is an increase in the total protein of the blood.
4. There is usually a moderate to severe normocytic anemia.

VITAMIN-C DEFICIENCY

Scurvy:

This disease is due to a deficiency of vitamin C and is characterized by subperiosteal hemorrhages, purpura, gingivitis, debility, irritability, anemia, generalized aches, and gastrointestinal disturbances.

Laboratory Examinations:

1. The ascorbic acid in the plasma is reduced. (The normal range is 0.5 to 0.7 mg. per 100 cc.)
2. The excretion of ascorbic acid in the urine is reduced. (The normal is 10 mg. in 24 hours.)
3. The saturation test shows evidence of tissue depletion of vitamin C. An adult is given 600 mg. of ascorbic acid and a child is given 70 mg. per 6.4 (14 pounds) of body weight. In a normal person, there is a considerable rise in urinary excretion in the next twenty-four hours; and in a deficient person, there is very little or none, depending on the state of unsaturation of the tissues.
4. Capillary fragility is increased as evidenced by a positive tourniquet test.
5. Hypochromic normocytic anemia is seen in adults. Platelets are normal.
6. Bleeding and coagulation times are usually normal. In severe cases, however, either or both may be prolonged.
7. Oliguria is seen commonly.
8. Hematuria with albuminuria is frequent. Hyaline, granular, or red-cell casts are present.

VITAMIN-D DEFICIENCY

Rickets:

Rickets is a disease of infants and children due to deficiency of vitamin D, which causes abnormal calcium and phosphorous metabolism with consequent defective ossification. Various deformities develop, such as knock knees, bow legs, enlargement of the epiphyses, scoliosis, rachitic rosary, and bosses on the temporal bones. Dentition is usually delayed.

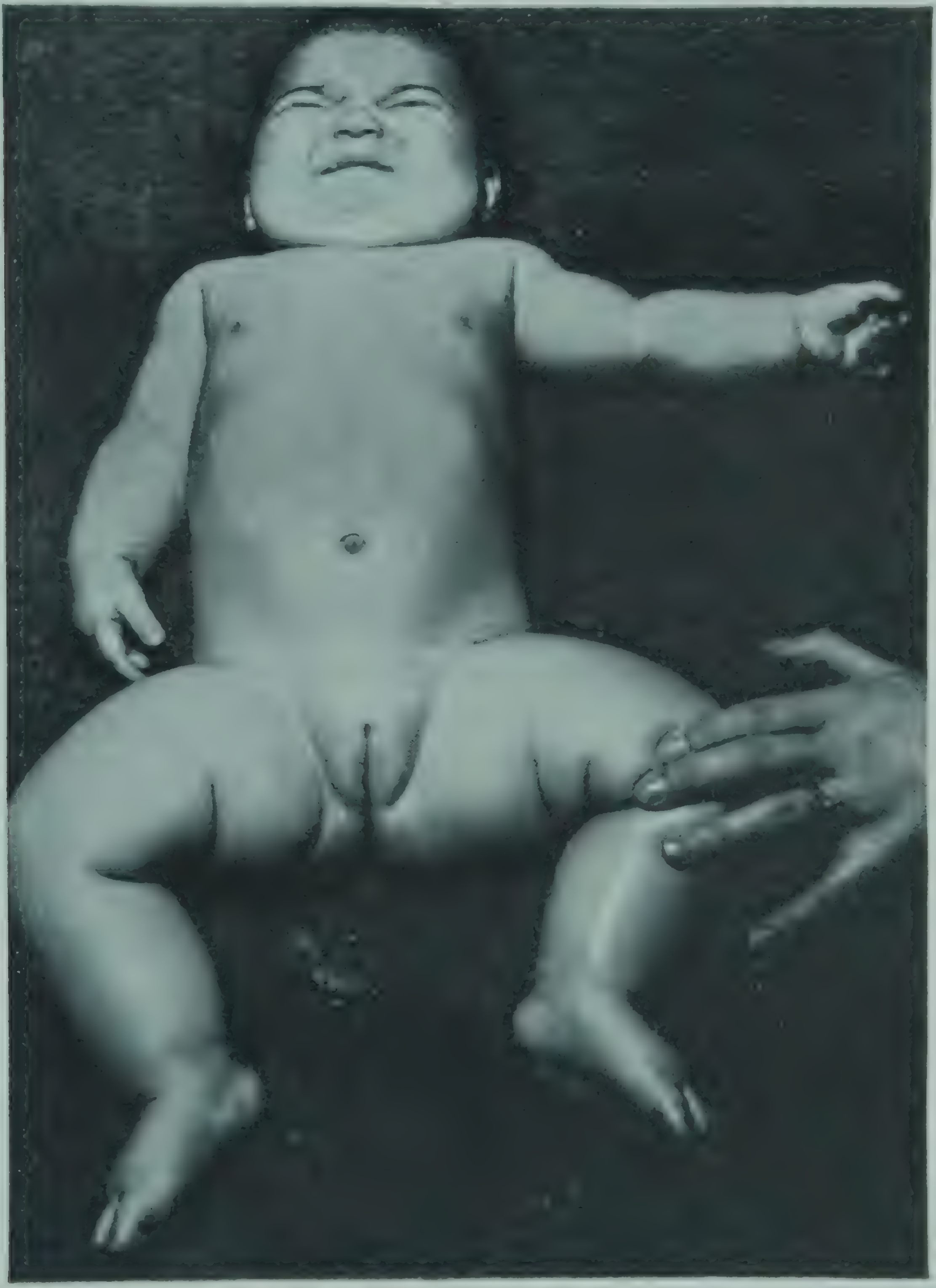

Fig. 1: Scurvy in infant. Note characteristic position of legs and swollen extremities.

Laboratory Examinations:

1. Serum calcium is decreased below the normal range of 9 to 11 mg. per 100 cc. when tetany is present. It may be normal in some cases.

2. Serum phosphate is decreased below the normal range of 4 to 7 mg. per 100 cc.
3. Alkaline serum phosphatase is increased above the normal range for children of 4 to 14 Bodansky units per 100 cc. It is due to increased osteoblastic activity.
4. The red cells show a hypochromic normocytic anemia, but it may be macrocytic in some cases.
5. Urinary calcium is decreased, but fecal calcium is increased.
6. There is no laboratory test for the estimation of vitamin D in body fluids.

VITAMIN-K DEFICIENCY

Vitamin K performs an important function in the formation of prothrombin in the liver by a mechanism that is at present unknown.

Sources of Vitamin K:

There are two sources of vitamin K: (1) food (cabbage, cauliflower, spinach, bran, and alfalfa) and (2) bacteria of the lower intestine. The vitamin K formed by the bacteria of the lower intestinal tract is believed to be sufficient for the mimimal requirements of man.

Absorption of Vitamin K:

Vitamin K is oil-soluble and requires bile for its absorption. After it is absorbed into the bloodstream, it is carried to the liver where it participates in the manufacture of prothrombin.

Hypoprothrombinemia:

This condition may result from the following: (1) an inadequate amount of vitamin K because of inadequate intake or sterility of the intestine, as in the newborn; (2) absence of bile from the intestine, as in obstructive jaundice or biliary fistula; (3) inability of the liver to manufacture prothrombin because of destructive liver disease, such as hepatitis, acute yellow atrophy, or cirrhosis; and (4) certain intestinal diseases, such as ulcerative colitis and sprue, which interfere with absorption of vitamin K.

Laboratory Examinations: At present, there is no laboratory method for determining the level of vitamin K in the blood. The prothrombin time and index[8] may be determined as an indirect means of determining the presence of vitamin K. However, if vitamin-K therapy does bring about a normal prothrombin time in a nonjaundiced patient, it must be inferred that liver damage is present. In fact, the prothrombin index before and after parenteral vitamin K is a means of determining parenchymal liver disease. See page 235.

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CHAPTER 29

Metabolic Disorders

PATHOLOGICAL CHEMISTRY OF BODY FLUIDS

ELECTROLYTE AND FLUID BALANCE

A knowledge of the physiology of electrolyte and fluid balance is necessary for the proper diagnosis and management of postoperative patients and such conditions as shock, burns, dehydration, acidosis, and alkalosis.

Physiology:

The sources of body water are as follows: (1) the liquids taken orally, (2) the fluid content of the solid foods, and (3) water produced by the oxidation of material within the tissues. The channels of loss of water are as follows: (1) expired air, (2) sweat, (3) urine, (4) feces, and (5) tears and saliva (insignificant amount).

Abnormal water loss may take place as follows: by vomiting, up to 5 liters; overventilation, 1 to 2 liters; excessive sweating, 10 to 15 liters; diarrhea, up to 5 liters; and polyuria in diabetes insipidus, up to 30 liters or more in twenty-four hours.

Water excretion by the body through any channel except the lungs is invariably accompanied by electrolytes, chiefly sodium and chloride. Loss of water by expired air, sweat, and feces is obligatory, while the kidneys excrete only that water that remains after losses by the first three routes have been satisfied.

Body fluids are divided between the intracellular and extracellular compartments. Extracellular fluid consists of circulating blood and interstitial fluids, which include the lymph. The circulating blood plasma constitutes 5 per cent of body weight, or about 3.5 liters in a person weighing 68.1 kg. (150 pounds); the interstitial fluid is 15 per cent of body weight, or 11 liters; and the intracellular fluid is 50 per cent of body weight, or 35 liters.

The volume of interstitial fluid is the most variable because of the ability to expand and contract with changing physiological needs. In fact, it may undergo extensive change that is not reflected in the volume or constituents of circulating blood. There is a constant exchange of fluid between the blood and the interstitial spaces, but in spite of this the volume of circulating blood is relatively constant. The volume of the plasma is affected by hydrostatic pressure, plasma-protein concentration, osmotic pressure of interstitial fluid, available water, and the total concentration of solutes in the extracellular fluid. The maintenance of normal composition of all body fluids is largely a function of the kidneys.

Electrolyte Distribution:

The normal electrolyte distribution of plasma, as listed by Hawk, Oser, and Summerson,[1] is shown below. These values are subject to wide variation under both normal and pathological conditions:

APPROXIMATE ELECTROLYTE DISTRIBUTION OF NORMAL HUMAN BLOOD PLASMA

	Bases	=	*Acids*
Ion	Na + K + Ca + Mg	=	Cl + HCO_3 + Pr + Others
Concentration*	154 + 5 + 5 + 3	=	106 + 28 + 17 + 16
	167	=	167

The relative amounts of electrolytes in the various body fluids are shown graphically in the diagram made by Gamble.[2] It will be noted that plasma differs from interstitial fluid only in containing protein (Fig. 1).

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* Milliequivalents per liter of plasma water.

ACID-BASE EQUILIBRIUM

In the metabolic processes of the body, phosphoric, sulfuric, hydrochloric, carbonic, lactic, and organic acids are found continuously. However, the mechanisms that are called into play keep the *p*H of the blood remarkably close to 7.4 by neutralizing and removing excess acid.

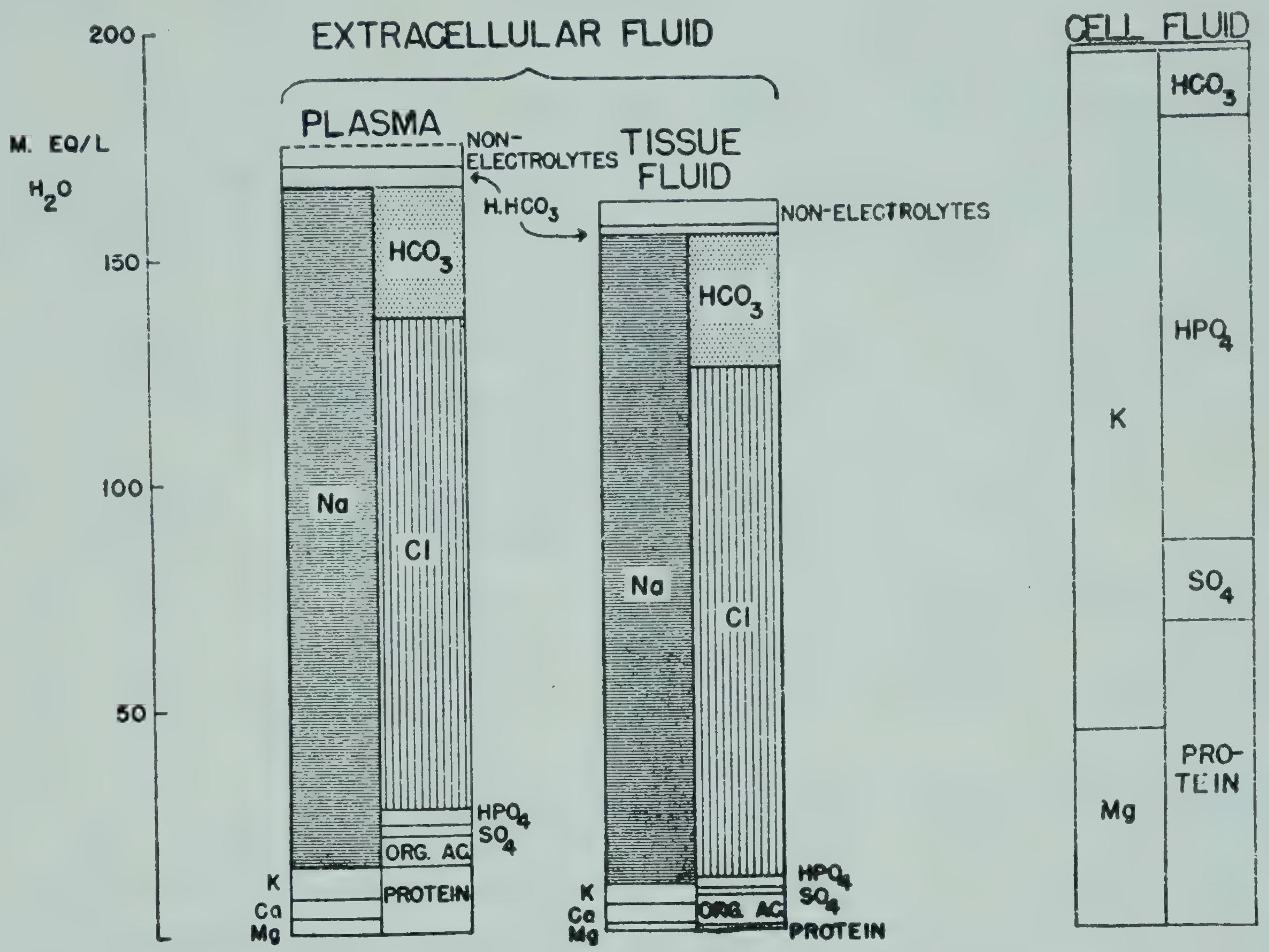

Fig. 1: Composition of body fluids. (From Gamble, J. L.: Chemical Anatomy, Physiology, and Pathology of Extracellular Fluid. Lecture Syllabus, Department of Pediatrics, Harvard Medical School.)

The *p*H of the blood is kept normal by the following mechanisms:

1. Buffer systems of blood
2. Excretion of carbon dioxide by lungs
3. Excretion of fixed acids by kidneys
4. Removal of part of phosphoric acid by intestinal mucosa

Buffer Systems of Blood:

The buffer systems[3] of the blood are as follows:

Primary Plasma Buffers:

1. Free carbonic acid and acid bound as sodium bicarbonate with a ratio of 1:20:

$$\frac{H_2CO_3}{NaHOC_3} = \frac{1}{20}$$

2. The acid and alkaline phosphates of sodium (ratio 1:5):

$$\frac{\text{Acid sodium phosphate}}{\text{Alkaline sodium phosphate}}\left(\frac{NaH_2PO_4}{Na_2HPO_4}\right) = \frac{1}{4}$$

3. Plasma proteins, which at the reaction of the blood behave as acids and so combine with base:

$$\frac{\text{H protein}}{\text{Na proteinate}}$$

Secondary Buffers in Erythrocytes:

1. Oxyhemoglobin and reduced hemoglobin each act as a weak acid of a buffer pair of which the potassium salt of the pigment acts as the other half as follows:

$$\frac{\text{H oxyhemoglobinate}}{\text{Potassium oxyhemoglobinate}}\left(\frac{HHbO_2}{KHbO_2}\right)$$

and

$$\frac{\text{H hemoglobinate}}{\text{Potassium hemoglobinate}}\quad\left(\frac{HHb}{KHb}\right)$$

2. The potassium salts of phosphoric acid:

$$\frac{\text{Acid potassium phosphate}}{\text{Alkaline potassium phosphate}}\left(\frac{(KH_2PO_4)}{(K_2HPO_4)}\right)$$

Excretion of Carbon Dioxide by Lungs:

The sensitivity of the respiratory center brings about rapid adjustments of the ratio H_2CO_3 $NaHCO_3$. The slightest reduction in plasma bicarbonate is met by increased pulmonary ventilation with elimination of carbon dioxide. When excessive amounts of carbon dioxide are removed by forced breathing, a movement of chloride and bicarbonate ions occurs, and the plasma bicarbonate is reduced.

Excretion of Fixed Acids by Kidneys:

Base is conserved in the body by retention of part of the base in combination with organic acids. The organic acids are free in combination with fixed base or in combination with ammonia. Another method by which fixed base is saved by the kidneys is in the manufacture of ammonia for the neutralization of acids. The kidney separates certain acid radicals from the bases with which they were combined in the plasma, combines them with ammonia, and excretes them as salts of ammonia.

Dehydration:

Dehydration may result from water loss or salt depletion, but more commonly it is a mixed water-and-salt loss. As a result, the body water and salt are reduced below normal, but water reduction is relatively greater. Loss of plasma water is usually made up by the passage of interstitial fluids into the blood. The interstitial fluid has a wide range of adaptability in defense of plasma fluid.

In dehydration, there is not only change in the electrolyte composition but also in the acid-base balance of plasma and interstitial fluids, usually toward the acid side. The diminished quantity of circulating fluid (anhydremia) and the consequent depression of oxidative processes in the tissues are thought to be responsible for the excessive production of acid metabolites, for example lactic acid. The slowing of the renal circulation also leads to a reduced excretion of urine and the retention of acids, such as phosphoric acid, which are normally excreted.

The clinical estimate of the state of dehydration seems to be the best guide to therapy, but certain laboratory tests are helpful.

Laboratory Examinations:

1. Urine chlorides are reduced or absent. Normal excretion is 10 to 16 gm. in twenty-four hours.
2. Plasma chloride is reduced. Normal plasma chloride is 550 to 650 mg. per 100 cc.
3. Plasma, carbon-dioxide combining power is reduced. Normal is 55 to 65 volumes per cent.
4. Total protein is increased.
5. Hematocrit is increased.

Disturbance of Acid-Base Equilibrium:

Acidosis is usually used to indicate a decrease in the alkali reserve (plasma bicarbonate) below the normal level. Since the bicarbonate represents the base in the blood that is left over after the nonvolatile acids have been neutralized, a reduction in alkali reserve frequently indicates—though not necessarily—that an excess of fixed acids is being produced or retained in the body.

Alkalosis is used to indicate an increase in alkali reserve, which may or may not be associated with a rise in blood *p*H.

Van Slyke[4] prefers to use the terms "alkali deficit," or "carbon-dioxide excess," and "alkali excess," or "carbon-dioxide deficit," instead of "acidosis" and "alkalosis." He recognizes one normal and eight abnormal states. In any one of these, the ratio $H_2CO_3/NaHCO_3$ may be increased or diminished with consequent elevation or depression of *p*H; the acid-base condition is then said to be "uncompensated." However, when adjustments occur to maintain the ratio at the normal value, the condition is said to be "compensated."

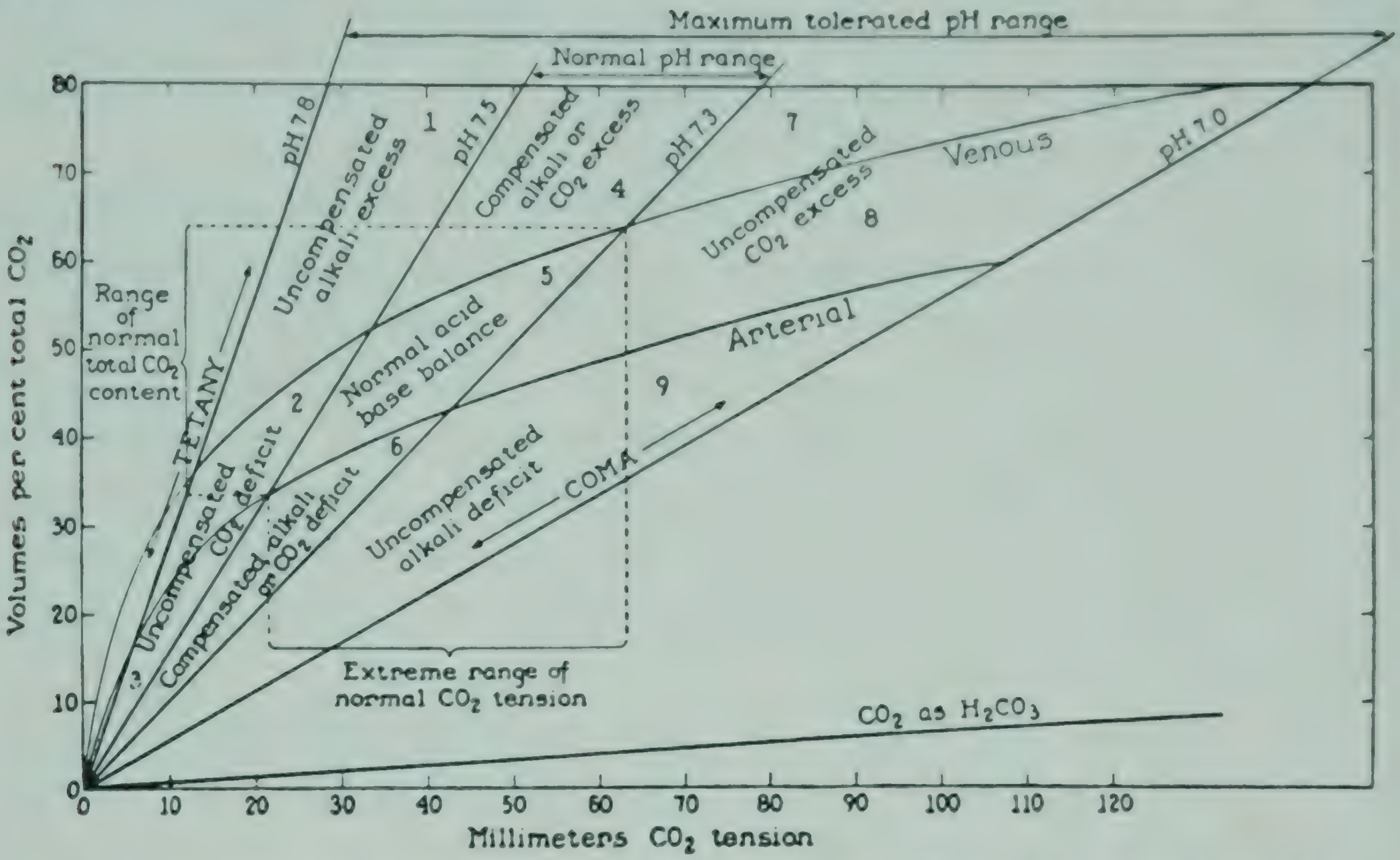

Fig. 2: Normal and abnormal variations of the $BHCO_3$, H_2CO_3, CO_2 tension, and *p*H in oxygenated human whole blood drawn from resting subjects at sea level.

The curved lines are the CO_2 dissociation curves of reduced (upper curve) and oxygenated blood (lower curve). The straight slanting lines indicate different $NaHCO_3/H_2CO_3$ ratios, the *p*H at each ratio having been calculated from the Henderson-Hasselbalch equation. The CO_2 present as bicarbonate at any point is obtained by subtracting from the total CO_2 the relatively small amount present as H_2CO_3, indicated by the line near the bottom of the chart. The ratio, and so the *p*H, is the same at all points along a given line. Thus, the intersection of the CO_2 dissociation curves by these so-called "isohydrionic lines" marks off nine areas corresponding to the acid-base states described in the text. (From Hawk, P. B., Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry. The Blakiston Company, Philadelphia, 1947.)

The acid-base states described by Van Slyke[4] are as follows:

1. Uncompensated alkali excess: In this condition the sodium bicarbonate is increased without a proportionate rise in carbonic acid. The *p*H is raised (that is the alkalinity of the blood is increased). This may result from the ingestion of large quantities of alkali (sodium bicarbonate) or from the loss of hydrochloric acid by vomiting, as in pyloric obstruction (area *1*, Fig. 2).
2. Uncompensated carbon-dioxide deficit: In this, the carbonic acid is reduced below normal, but the sodium bicarbonate is not lowered to a corresponding extent. The *p*H is raised. A disturbance of this nature may result from forced breathing or the hyperventilation induced by hot baths, high altitudes, or shallow breathing. It is sometimes also referred to as "gaseous alkalosis." Partial compensation occurs, as evidenced by the reduced excretion of acid and ammonia in the urine and the increase in the urinary bicarbonate (areas *2* and *3*, Fig. 2).
3. Compensated alkali excess: The sodium bicarbonate is raised, but a parallel rise occurs in carbonic acid. The *p*H is therefore normal. A disturbance of this nature will result from conditions that cause pyloric obstruction (area *1*, Fig. 2) but of less severe degree (area *4*, Fig. 2).
4. Compensated CO_2 excess: The carbonic acid is increased but is accompanied by a proportional rise in sodium bicarbonate; *p*H is normal. This state occurs in conditions in which there is retention of carbon dioxide, for example emphysema (area *4*, Fig. 2).
5. Normal acid-base balance: Carbonic acid, sodium bicarbonate, and *p*H are normal (area *5*, Fig. 2).
6. Compensated alkali deficit: Sodium bicarbonate is reduced and carbonic acid diminished proportionately; *p*H is normal. The condition results from the abnormal production of fixed acids, as in diabetes; acid retention, as in nephritis; or from the ingestion of mineral acids or acid-producing salts (calcium chloride or ammonium chloride). This and area *9* in Fig. 2 are the acid-base states to which the term "acidosis" has usually been applied. The compensatory adjustments are evident in the increased acid and ammonia excretion in the urine and the lowered carbon-dioxide tension in the alveolar air. Alkali deficit may also result from the increased excretion of base as occurs in dehydrated states and in animals following the production of a pancreatic fistula (area *6*, Fig. 2).
7. Compensated carbon-dioxide deficit. This is a reduction in carbonic acid and a parallel reduction in sodium bicarbonate.

The pH is therefore normal. This state results from less severe grades of the conditions that cause uncompensated carbon-dioxide deficit (paragraph 2). The excretion of sodium bicarbonate by the kidney is much slower than the excretion of carbon-dioxide by the lungs; therefore, a condition of carbon-dioxide deficit that is uncompensated to start with later tends to be compensated (area *6*, Fig. 2).

8. Uncompensated carbon-dioxide excess: Carbonic acid is increased but is not balanced by a proportional rise in sodium bicarbonate. The pH is lowered (that is blood is less alkaline). This is sometimes referred to as "gaseous acidosis." Such a state results from a hindrance to the excretion of carbon dioxide, as may occur in pneumonia, obstruction to breathing, or depression of the respiratory center by morphine. There is partial compensation, that is the bicarbonate is raised above the normal and there is an increased excretion of acid and ammonia by the kidneys (areas *7* and *8*, Fig. 2).
9. Uncompensated alkali deficit: Sodium bicarbonate is reduced without there being a parallel reduction in carbonic acid. The pH is lowered (that is the blood becomes less alkaline). Such an acid-base state occurs when large quantities of fixed acids (betahydroxybutyric and acetoacetic) are produced, as in diabetic coma and in the terminal stages of nephritis when acid excretion is greatly impaired. The plasma bicarbonate is severely depleted, and the carbon-dioxide combining power of the plasma may be less than 20 volumes per cent (area *9*, Fig. 2).

Acidosis (see also p. 59). LABORATORY EXAMINATIONS:

1. The carbon-dioxide combining power is reduced. Depending on the severity of acidosis, the values range from 50 volumes per cent (mild acidosis) to 20 or even 10 volumes per cent (severe acidosis).
2. The carbon-dioxide tension of the alveolar air is reduced from the normal range of 5.2 to 5.7 volumes per cent (not usually employed).
3. The pH of the blood may or may not be reduced (not usually employed).
4. Severe diabetes and diabetic coma are the most common conditions in which acidosis is due to the production of ketone bodies that are usually found in the urine. They are acetone, diacetic acid, and betahephoxybutyric acid.
5. Blood calcium may be reduced.

Alkalosis (see also p. 59). LABORATORY EXAMINATIONS:

1. The carbon-dioxide combining power is increased. Depending on the severity of the alkalosis, the values range from 70 volumes per cent (mild alkalosis) to over 100 volumes per cent (severe alkalosis).
2. The carbon-dioxide tension of the alveolar air is increased from the normal range of 5.2 to 5.7 volumes per cent (not usually employed).
3. The *p*H of the blood may or may not be increased (not usually employed).
4. The urine may show acetone even in alkalosis.

EDEMA

Edema is the accumulation of fluid in the interstitial spaces. The following factors control the exchange of fluid between the blood and interstitial spaces:

1. Hydrostatic pressure in the capillaries (normal: 13 to 35 mm. Hg)
2. Plasma colloid osmotic pressure dependent largely upon the concentration of albumin (normal: 25 to 30 mm. Hg); the critical level below which edema occurs is 20 mm. Hg
3. Capillary permeability to protein
4. Lymphatic circulation

Landis[5] summarizes the changes that are concerned in the etiology of edema as follows:

1. Increased capillary pressure (hydrostatic pressure)
   a. External pressure on veins
   b. Thrombophlebitis
   c. Congestive heart failure
   d. Dependency
2. Reduced colloid osmotic pressure
   a. Loss of albumin
   b. Inadequate protein intake
   c. Impaired synthesis of protein
   d. Sudden plasma dilution
3. Increased capillary permeability
   a. Inflammation
   b. Nephrosis
   c. Anemia
   d. Chronic anoxemia

4. Lymphatic obstruction
    a. Lymphedema
    b. Increased venous pressure

Laboratory Examinations:

Laboratory investigations are of value only in the type of edema resulting from reduced, colloid, osmotic pressure, except as they may aid in the diagnosis of the underlying disease.

1. Plasma total protein is reduced to 5 Gm. or less per 100 cc. A level of 5 Gm. per 100 cc. is the point at which edema usually begins.
2. Plasma albumin is reduced 2.5 Gm. or lower per 100 cc. A level of 2.5 Gm. of albumin is the point at which edema usually begins.
3. Specific gravity is reduced to the critical level for edema formation, namely 1.023 (normal average is 1.027).

SHOCK

Shock is a state of collapse characterized by fall in blood pressure, pallor, coldness of skin, cyanosis of finger tips and lobes of ears, sweating, fall in body temperature and of metabolic rate, rapid shallow breathing, rapid thready pulse, and apathy. The current theories as to the cause of wound shock are as follows: (1) a toxic agent is formed in the injured tissues, is carried in the bloodstream, and exerts a widespread effect on the vascular system; and (2) circulating fluid is lost into the damaged parts.

Laboratory Examinations:

1. Hemoconcentration is present and is evidenced by increase in the hematocrit and erythrocyte count.
2. The blood volume is reduced.
3. The carbon-dioxide combining power (alkali reserve) is reduced.
4. The nonprotein nitrogen is increased.

BURNS

The result of extensive burns brings about changes in the blood that are helpful in determining the therapeutic needs of the patient.

Laboratory Examinations:

1. Hemoconcentration is marked in burns because of the loss of serum. The hematocrit and erythrocyte count are increased in proportion to the serum loss.

2. The total protein is increased.
3. The blood volume is reduced.
4. The blood viscosity is increased.
5. A month or more after the burn, a secondary anemia develops.
6. According to Cope and associates,[6] the 17-ketosteroid excretion is high-normal or increased during the first three to seven days. Following this, there is a precipitous drop to low-normal or subnormal excretion. The relation of 17-ketosteroid excretion to nitrogen metabolism and to adrenal-cortical activity is not settled.
7. According to the same authors,[6] there is a moderate, negative-nitrogen balance in many patients in the first week.

METABOLIC DISORDERS

Glycogen-Storage Disease

Glycogen-storage disease, also known as "von Gierke's disease,"[7] is a congenital metabolic disorder characterized by the storage of large amounts of glycogen in the liver, kidneys, heart, striated muscle, and various other organs. The cause is not known, but it is an inherited metabolic disorder. The following theories have been suggested: (1) the disease is due to lack of glycogenase in the liver; (2) the glycogen in the liver and other organs is in abnormal combination with proteins; or (3) it may be due to some abnormal function of the anterior pituitary gland. The glycogen in the various organs is very stable and is not readily converted into glucose. There is consequently a constant hypoglycemic state.

Laboratory Examinations:[8]

1. The fasting blood glucose is decreased and is frequently as low as 50 mg. per 100 cc.
2. In the glucose-tolerance test, there is a delayed drop in the curve.
3. The blood glucose does not increase over 30 mg. above normal in fifteen to thirty minutes following the subcutaneous injection of 0.5 cc. of 1:1000 solution of epinephrine. (The normal rise is over 30 mg.)
4. The blood-sugar level rises within a half hour after the administration of levulose.
5. According to Hogg and Sidbury,[9] the total lipids of the blood are increased, especially cholesterol.
6. The morning specimens of urine are constantly positive for acetone.

MISCELLANEOUS MELITURIAS

Melituria is defined as the presence of any sugar in the urine, including glucose. The other sugars that may occur in the urine are galactose, lactose, pentose, and levulose. These are discussed in Chapter 4 (p. 134). The ordinary reduction tests do not differentiate the sugars mentioned above; thus, special tests must be carried out when a sugar other than glucose is suspected. Needless to say, in all meliturias, it is important to rule out diabetes mellitus.

Renal Glycosuria:

Renal glycosuria is a benign type of glycosuria in which the kidney threshold for glucose is below normal, but the blood sugar level is normal. True renal glycosuria includes only the continuous type, that is when glucose occurs in the urine in both the fasting state and after meals. The importance of the condition lies in differentiating it from diabetes-mellitus glycosuria.

The cause of renal glycosuria is not known, but at present it appears that the low renal threshold is the result of incomplete reabsorption of glucose by the kidney tubules.

The diagnosis of renal glycosuria and its differentiation from diabetes mellitus are based entirely upon laboratory examinations. A person with renal glycosuria should be kept under observation indefinitely lest diabetes mellitus should develop at any time.

Laboratory Examinations:

1. The urine is positive for glucose after meals in the intermittent type, and at all times in the persistent, or continuous, type of renal glycosuria.
2. The fasting blood glucose is normal at all times.
3. The glucose-tolerance test is particularly helpful in ruling out diabetes mellitus. The blood-sugar curve is normal in renal glycosuria; but in spite of this, glucose is found in all urine specimens taken with the test.
4. In contrast to diabetes mellitus, acidosis does not develop after excessive ingestion of food.

ALKAPTONURIA

This condition is an abnormality of tyrosine and phenylalanine metabolism in which homogentisic acid is excreted in the urine. The cause is unknown, but it is a familial disorder in which the person is unable to carry tyrosine beyond the stage of homogentisic-acid formation. Feeding of tyrosine or phenylalanine to a patient with alkaptonuria increases the output of homogentisic acid. If fed homogentisic acid, it is excreted unchanged, whereas the normal person is able to oxidize homogentisic acid completely.

Laboratory Examinations:

1. The condition is first suspected in infants because of a dark brown stain on the diapers.
2. The urine turns dark brown in the presence of alkali and oxygen.

PORPHYRIA

The occurrence of porphyrins in the urine is characteristic of a rare disease of pigment metabolism called "porphyria." It is discussed in Chapter 4 (p. 138).

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CHAPTER 30

Collection and Care of Laboratory Specimens

HEMATOLOGY

BLOOD COUNTS

Because of the normal error in blood counts, it is most important to have the best of pipets and counting chambers and to keep them in perfect condition. Broken tips and coagulated blood in the bulbs contribute to inaccurate results. Further, the blood and diluting fluids must be drawn accurately to the appropriate lines. For best results, the following procedures should be carefully carried out:

1. The lobe of the ear or ball of the ring finger may be used in adults. The side of the tip of the finger is perferred by some beause it is less painful. In infants, it is necessary to use the toe or the heel.
2. Refrain from squeezing unduly because an excess of tissue juices may be forced out, thus causing inaccurate results. The finger or ear lobe should be rubbed to promote circulation.
3. The skin is cleaned with 70 per cent alcohol and is allowed to dry before pricking.
4. Hold the part firmly, and prick it deeply enough to allow adequate bleeding. This should be done with an instrument with a wide cutting edge, such as a Hagedorn needle.

5. The first drop of blood is wiped off.
6. From a drop of blood of adequate size, the pipet is filled exactly to the proper mark, and the remainder is filled with appropriate diluting fluid. It is then mixed thoroughly. To prevent leakage of the contents, the pipet is protected by folding the rubber tube over the ends or by applying a rubber band.
7. Blood smears are made on slides or cover slips that are clean and free of all fatty material. If there is to be any delay in staining, they should be protected from flies, which will ruin a smear by ingesting the blood.
8. It is advisable to prepare both thick and thin blood smears for the examination for malarial parasites. The thick smear is prepared by placing four drops of blood in the form of a square. They are mixed together with a toothpick or the corner of another slide and allowed to dry. Staining with diluted Giemsa's stain results in hemolysis of the erythrocytes and staining of Plasmodia, leukocytes, and platelets.
9. It must be remembered that reticulocytes do not take the ordinary blood stains. They are stained with a vital stain, such as brilliant cresyl blue. A practical procedure for the doctor's office is to use cover slips on which brilliant cresyl blue has been previously dried and partially rubbed off. Coverslip preparations are made in the usual manner and counterstained with Wright's stain.

BLOOD CHEMISTRY

For uniform results, which can be used for comparison with subsequent values, blood should be drawn before breakfast. The filtrates should be prepared within two hours at the latest. This is particularly true in hot climates and in the heat of summer when glucose levels decrease because of glycolytic enzymes. Blood specimens may be preserved in an icebox at 39.2° F. (4°C.) when they cannot be processed immediately.

Since whole blood or plasma is used for many chemical tests (see p. 46), it is necessary to prevent clotting. This is done by the use of an anticoagulant, such as sodium or potassium oxalate or citrate, which removes the ionized calcium. One to 2 mg. of neutral potassium oxalate is required per cubic centimeter of blood. Only an amount of anticoagulant sufficient for the quantity of blood to be drawn should be used. Too much anticoagulant may interfere with some analyses, may cause hemolysis, or may produce an abnormal distribution of electrolytes and water between cells and plasma.

Preparation of Oxalate Tubes:

Prepare a stock solution of 10 per cent neutral potassium oxalate and place 0.1 cc. in each tube. Rotate and dry at not over 176° F. (80° C.) because higher temperatures convert part of the oxalate to carbonate with production of blood clot when blood is placed in the tubes. This amount of oxalate is sufficient to prevent clotting of 6 to 10 cc. of blood. For 3 to 5 cc. of blood, use half the amount of anticoagulant.

Other Anticoagulants:

Following is a list of other anticoagulants with the amount required per cubic centimeter of blood:

Anticoagulant	*Amount in Mg. per Cc. of Blood*
Sodium oxalate	1 to 2
Lithium oxalate	1 to 2
Sodium citrate	5
Sodium fluoride	10
Heparin	0.2

A special mixture suggested by Heller and Paul,[1] containing three parts of ammonium oxalate and two parts of potassium oxalate, has the advantage of causing no change in erythrocyte volume. It is useful for hematocrit, sedimentation, and specific-gravity determinations. It cannot be used in ordinary analytical procedures because of the presence of ammonia.

Prothrombin Determination:

In collecting blood for prothrombin determination (prothrombin time and prothrombin index), the proper anticoagulant, according to Quick,[4] is 1.34 Gm. of anhydrous pure sodium oxalate in 100 cc. of distilled water. Five tenths of a cubic centimeter (0.5 cc.) of this anticoagulant is mixed with 4.5 cc. of blood.

Preservation of Glucose:

Sodium fluoride is a preservative and inhibits the glycolytic decomposition of glucose. According to Irish, Boyd, and Roe[3] 10 mg.

of sodium fluoride per cubic centimeter of blood will preserve glucose, nonprotein nitrogen, uric acid, creatinine, and cholesterol for ten days, provided the specimen is sterile. It is thus a practical preservative for mailing specimens.

Preparation of Serum:

Certain chemical procedures are carried out with serum (see p. 46). Place the blood in a test tube without an anticoagulant, and allow to clot at room temperature. Chill in the refrigerator, centrifuge, and remove the serum with a rubber-bulb pipet.

If a centrifuge is not available, allow the blood to clot with the tube in a slanting position; then after chilling overnight in an upright position, the serum may be poured off from the side of the tube opposite the clot.

SERUM

1. Blood for serological tests is prepared as described above. It is desirable to keep the specimens sterile because bacterial growth interferes with subsequent procedures.
2. It is important that hemolysis be avoided because this interferes with certain tests. Hemolysis can be avoided by using a dry syringe, needle, and tube.
3. If the blood is to be mailed, it is advisable to send only the serum, especially if the transportation will take longer than two days.

BLOOD CULTURES

1. Complete asepsis must be maintained in collecting the blood. As a general rule, the inoculation of 100 cc. of appropriate broth with 5 cc. of blood is satisfactory. It is sometimes advisable to inoculate two flasks of media in order to incubate one aerobically and one anaerobically.
2. It is advisable for the clinician to check with the laboratory as to the use of agents in the media that will neutralize the sulfonamides and penicillin. The specimen of blood may contain enough of the therapeutic agent to prevent the growth of bacteria. *Para*-aminobenzoic acid (5 mg. per 100 cc. of media) is used to neutralize sulfonamides. Penicillinase (10 units, or 0.1 cc. to each 15 cc. of media) is used to neutralize penicillin. At the present writing, no agent has been publicized that will neutralize streptomycin or the other antibiotics.

URINE

Routine Examinations

1. It is important to use carefully cleaned containers for urine specimens so that albumin and glucose from a previous specimen will not falsify the current specimen. Further, for females, it is necessary to use thoroughly cleaned bedpans. Feces must not be mixed with urine because this renders the tests invalid. I have seen ova of intestinal parasites in urine because a bedpan had not been thoroughly cleaned of previous fecal contents.
2. The evening specimen or the specimen passed two or three hours after a meal is more likely to contain albumin or sugar than the morning specimen.
3. Quantitative examinations should in general be carried out on the twenty-four hour excretion.
4. Vaginal discharge (leukorrhea) and menstrual discharge should be avoided in collecting specimens because of the obvious false findings in the sediment.

Bacteriological Examinations

1. Urine for culture should be secured by catheterization directly into a sterile container. This should be covered with a sterile cap or paper with or without sterile gauze. Gauze alone is not complete protection against the entrance of bacteria.

   When conditions prevent catheterization of the male patient, a specimen may be passed directly into a sterile container after thorough cleaning of the head of the penis.
2. Cystoscopy specimens for culture should be collected directly into sterile containers, which are immediately covered. These specimens are, of course, properly labeled "left kidney," "right kidney," and "bladder."
3. Specimens may be voided for examination for the Mycobacterium tuberculosis, although the Mycobacterium smegmatis may be a confusing contaminant in the stained smear.

Pregnancy Test

1. Collect the first specimen of urine passed in the morning in a thoroughly cleaned container. Bottles that have contained perfume or other chemicals may result in false-negative results.
2. The urine is kept in the refrigerator until used.
3. If the specimen is to be mailed, add about 200 mg. of boric acid or 100 mg. of thymol for 100 cc. of urine.
4. If the patient is bleeding from the vagina, she should be catheterized because human blood in the urine will kill the rabbit.

17-KETOSTEROIDS

1. Discard urine passed at 11 P.M., and collect all urine passed from 11 P.M. to 7 A.M.
2. Put urine in a container which has a few drops of toluene.
3. Repeat the procedure during the next night, and collect the urine in a separate container.
4. Give name, age, sex, date, and the diagnostic problem.

ESTROGENS

1. For estrogens, or estrogens and 17-ketosteroid determination, collect three, separate, twenty-four-hour samples of urine. Discard the urine passed before beginning the collection, and note the time.
2. Put each twenty-four-hour sample in a separate container which has a few drops of toluene.
3. Give name, age, sex, date, and the diagnostic problem.

GASTRIC AND DUODENAL CONTENTS

1. Each specimen should be sent to the laboratory for examination immediately after withdrawal.
2. The specimens should be labeled "fasting," "first specimen after test meal," and so on, in order that the results may be chronologically tabulated.
3. Any special tests desired should be listed.

CEREBROSPINAL FLUID

1. For routine examination, two test tubes, each containing 5 to 8 cc. of cerebrospinal fluid, are sent to the laboratory immediately. The cell count, smear for cells and organisms, culture, and quantitative glucose should be done immediately.
2. If a cell count cannot be done immediately, add 1 cc. of 10 per cent formaldehyde to 1 cc. of cerebrospinal fluid. This will preserve the cells, but correction must be made for the dilution when doing the count.
3. The glucose level can be preserved by keeping the specimen in the refrigerator or by adding thymol. However, in cases of meningitis, it is preferable to do the glucose determination immediately.
4. The presence of blood in cerebrospinal fluid prevents the accurate determination of a colloidal-gold curve.

SPUTUM

1. Collect a morning specimen that is free of saliva and nasal secretion. Material should be raised from the bronchi and collected in an appropriate container.
2. A twenty-four-hour collection is made for a concentration test for the Mycobacterium tuberculosis.
3. A sterile container is used when a culture is desired.
4. Specimens are sterilized before discarding.
5. The container should not be contaminated on the outside. This is a hazard to all who handle the specimen.

FECES

1. For routine examination, send a cubic inch of formed stool or an equivalent amount of liquid stool in an appropriate container. Routine examination usually consists of color, consistency, gross blood, occult blood, mucus, parasites, and microscopic examination for ova, fat, fatty-acid crystals, leukocytes, and erythrocytes. Urobilin is requested in cases of obstructive jaundice.
2. For culture, feces is passed into a sterile bedpan. From this, a small amount of feces with mucus, if possible, is taken up on a swab and dropped into a tube containing about 5 cc. of 50 per cent glycerin.
3. In ulcerative colitis, it is preferable to take a culture on a swab directly from the lesion and to drop it into the appropriate media.
4. In examining for the vegetative forms of amebas, the stool should be brought to the laboratory immediately so that they may be seen in motion. In transporting a specimen from a home, it is placed in a jar, and this is placed in another jar containing warm water. This precaution is not necessary for cyst forms.

TRANSUDATES AND EXUDATES

1. Fluids from the body cavities should be sent to the laboratory immediately so that cytologic studies can be made before the cells undergo degeneration.
2. Specify what examinations are desired.
3. A cell count of a transudate or exudate does not usually yield information of value. A differential count and cytological examination for tumor cells yield helpful information.

CULTURES AND SMEARS

1. Cultures and smears are taken carefully from the area involved. Indicate the source of the specimen.

2. When smears are prepared, make two smears so that different stains may be used if necessary.

CYTOLOGICAL STUDIES FOR TUMOR CELLS

The following instructions are given by Papanicolaou and Traut[4] for the preparation of vaginal smears for tumor cells:

1. Smears should not be taken after a douche or bath.

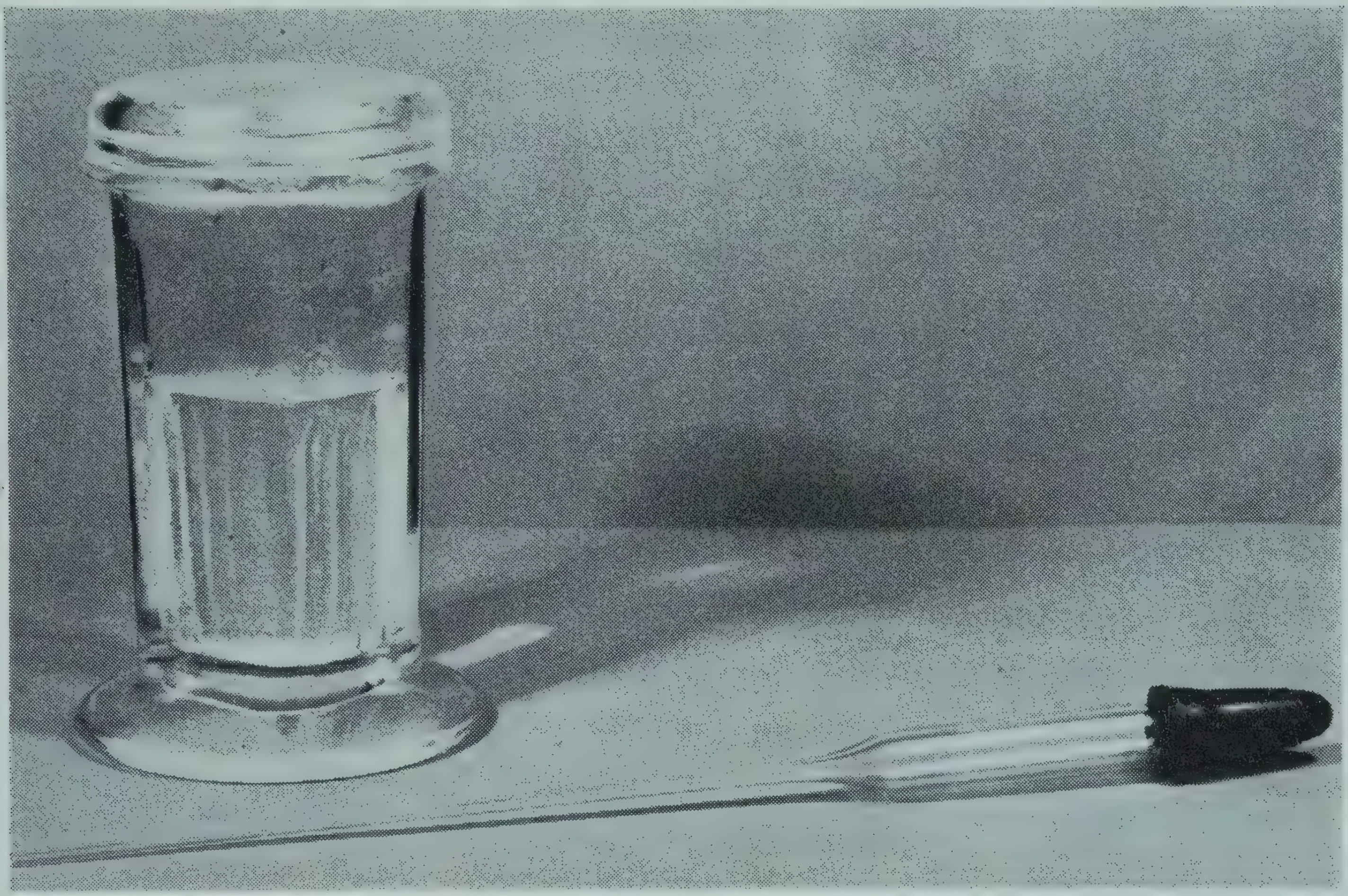

Fig. 1: Jar of fixative and pipet used in preparing vaginal smears for tumor cells.

2. Wipe off slides with a clean cloth, and have slides and solution ready before starting. Attach a paper clip to the end of each slide to separate the slides in solution and prevent the smears from touching each other.
3. First, press the bulb, and then insert the pipet into the vagina for about 7.6 to 10.2 cm. (3 to 4 inches). Release the bulb, and leave the pipet in the vagina for about one-half minute, moving it around cautiously. Remove the pipet slowly.
4. Blow the secretion on the slide with a sudden squeeze of the bulb, and smear it with the curved end of the pipet in order to secure an even distribution.

5. Drop the slide immediately into the solution (equal parts of ether and 95 per cent alcohol) with the clip attached and before the smear dries. Be sure to cork the bottle tightly to prevent evaporation and drying of smears.
6. The pipet must be cleaned by drawing cold and hot water through it several times. For sterilization, the pipet should be boiled or rinsed in alcohol or other antiseptic solution. Remove the bulb, squeeze all water out of it, allow the pipet to dry before using, and then replace bulb.
7. Do not keep the bottles near a radiator or flame, since the solution is inflammable.

The material for Papanicolaou stain may also be fixed by dropping it in a test tube containing 5 cc. of Bouin's solution. The technician then makes the smear from the sediment. This method has not been entirely satisfactory in my experience.

Smears for tumor cells from other material, such as sputum, bronchoscopic aspiration, chest fluid, ascitic fluids, urine, and so on, should also be fixed while moist.

SURGICAL SPECIMENS

1. Tissues should be processed as soon as possible so that they will be fixed before changes take place in the cells. To keep them moist from the time they are removed until they are fixed, they should be wrapped in gauze soaked in physiologic saline and then placed in wax paper. If a delay of more than a few hours is necessary, the specimens should be fixed in 10 per cent formaldehyde.
2. It is important to give name, age, source of tissue, brief history, ward or address, and name of the surgeon.

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